IDENTIFICATION OF FUNGAL SPECIES CAUSING POWDERY MILDEW ON CUCURBITS, DETERMINATION OF THE GENETIC AND PATHOGENIC VARIATIONS OF THE FUNGI AND THEIR SENSITIVITY TO MAJOR POWDERY MILDEW FUNGICIDES

BY

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DISSERTATION

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ABSTRACT

Cucurbit species are members of the family Cucurbitaceae. Major cucurbit species grown in Illinois are *Cucumis sativus* (cucumber), *Lagenaria sicerarias* (gourd), *C. melo* (muskmelon), *Cucurbita pepo* (pumpkin), *C. maxima* and *C. moschata* (squash), and *Citrullus lanatus* (watermelon). Powdery mildew is an annually-occurring disease of cucurbits, which causes yield losses up to 50% in Illinois and worldwide. *Podosphaera xanthii* and *Golovinomyces cichoracearum* are known as the causal agents of cucurbit powdery mildew. No reported investigations have been conducted on the species identification of the agent of powdery mildew of cucurbits, genetic and pathogenic variation of the pathogen, or the fungicide-resistance of the fungus in the North Central Region (NCR). This research was conducted to: 1) identify species causing powdery mildew on cucurbits in Illinois and some other major cucurbit producing areas in the United States; 2) determine genetic and pathogenic variations among isolates of powdery mildew fungi on cucurbits; and 3) determine the sensitivity of cucurbit powdery mildew pathogens to commonly used powdery mildew fungicides, including DMI, QoI, SDHI, quinolines, and cyflufenamid fungicides. To identify the species of powdery mildew fungi, powdery mildew infected leaves of *C. melo*, *C. sativus*, *C. maxima*, *C. moschata*, *C. pepo*, and *L. siceraria* were collected from California, Illinois, Indiana, Iowa, Michigan, Pennsylvania, Texas, Washington, and Wisconsin. In addition, 10 DNA samples from Italy and 40 isolates from New York and Wisconsin of cucurbit powdery mildew fungi were obtained. After sequencing the internal transcribed spacer (ITS) region of nuclear ribosomal DNA (nrDNA) for 119 isolates of powdery mildew causing fungi, only *P. xanthii* was identified. Distinct clustering of genetic variation based on geographical origins was found among the isolates. Also, significant variation ($P < 0.0001$) was found in pathogenicity among the 37 isolates tested. Our data showed reduced

effectiveness of azoxystrobin, from the QoI group, for the control of cucurbits powdery mildew. Triflumizole (DMI group), penthiopyrad (SDHI group), quinoxyfen (quinolines group), cyflufenamid (unknown mode of action group of U6) all effectively controlled powdery mildew of pumpkin.

To my families

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CHAPTER 1

INTRODUCTION AND OVERVIEW

Cucurbit production in the United States and in Illinois

Cucurbits refer to some plant species in the family Cucurbitacea. Cucurbits are among the earliest domesticated and cultivated plants in the world, and are grown in tropical and temperate regions. Cucurbits grown in the United States (US) are cantaloupe (*Cucumis melo*), cucumber (*Cucumis sativus*), jack-o-lantern pumpkin (*Cucurbita pepo*), processing pumpkin (*Cucurbita moschata*), watermelon (*Citrullus lanatus*), winter squash (*Cucurbita maxima*), yellow squash (*Cucurbita pepo*), and zucchini (*Cucurbita pepo*). According to the United States Department of Agriculture (USDA), 164,221 hectares were planted to cucurbits in 2016 in the US, which produced 4768,257 tons of fruit with a value of $1504 million. Among these, 28,490 hectares were planted to pumpkins in 2016 in the US, which produced a yield of 816,393 tons with a value of $208 million. Approximately 14,000 hectares of cucurbits are grown annually in Illinois, which includes about 4,000 hectares of jack-o-lantern pumpkins, 6,000 hectares of processing pumpkins (representing more than 90% of commercial processing pumpkins produced in the US), and 4,000 hectares of other cucurbits crops (Babadoost, personal communication; Islam and Babadoost et al., 2005; Jossey and Babadoost, 2008).

Powdery mildew of cucurbits

Fungal species

Powdery mildew on cucurbits is mainly caused by two species, *Podosphaera xanthii* (Castagne) U. Braun & N. Shishkoff (synonym: *Sphaerotheca fuliginea*) and *Golovinomyces*

cichoracearum var. *cichoracearum* (D.C.) V.P. Heluta (synonym: *Erysiphe cichoracearum*) (Miazzi et al., 2011). Although both species are important in Europe, *P. xanthii* was the only species has been found in the US samples tested by Lebeda et al. (2010).

Disease characteristics and crop losses

The most common symptoms of powdery mildew and signs of the pathogen on cucurbits are white colonies on the leaf surfaces, petioles, vines, and fruit stems (Perez-Garcia et al., 2009). The white colonies are composed of mycelia and asexual spores (i.e. conidia) (Glawe, 2008). Under favorable conditions, the colonies expand and merge together and cause chlorosis and early senescence of leaves. Severe early senescence of leaves leads to fruit sunscald and decreases the size and number of fruit produced. Early senescence of leaves can also result in decreased market value because of poor storability and flavor, caused by low soluble solids, poor rind color, and shriveled and discolored handles. For cantaloupe plants, advanced pathogen infection may result in decreased sugar content in fruit, which ultimately leads to decreased fruit quality and marketability. In other plants, such as cucumber, yellow squash, and zucchini, infection may directly cause reduced yield and quality. Powdery mildew is one of the most important limiting factors for cucurbit production worldwide (Perez-Garcia et al., 2009). Although great efforts have been devoted to study this disease and develop management methods, many basic aspects of the causal agents, such as genetic and pathogenic variations, have not been adequately investigated (Perez-Garcia et al., 2009).

Disease cycle

Powdery mildews are caused by biotrophic pathogens in the order *Erysiphales.* The causal agents of cucurbit powdery mildew grow on plant surfaces and absorb nutrients from the plant epidermal cells through haustoria. As biotrophic parasites, powdery mildew fungi cannot be cultured on nutrient media (Perez-Garcia et al., 2009). The life cycle of powdery mildews includes asexual stages (anamorphic) and sexual stages (teleomorphic) (Figure 1.1) (Glawe, 2008; Perez-Garcia et al., 2009).

In the asexual reproduction stage, hyphae and conidia are developed. Conidia production starts within a few days of infection of host plants. It usually takes from 3 to 7 days between the time of infection and the appearance of symptoms. Usually a large number of conidia are produced. Conidia are formed either singly or in a chain on specialized hyphae called conidiophores.

In the sexual reproduction stage, overwintering structures (chasmothecia) develop. Powdery mildew species may be heterothallic or homothallic. *Podosphaera xanthii* is a heterothallic fungus and requires two mating types to produce chasmothecia (Perez-Garcia et al., 2009). Typically, eight ascospores are produced in a sac-like ascus, and asci are enclosed in ascocarps with no natural opening. An increase in turgor pressure inside ascocarps causes them to split open and discharge their asci and ascospores. Appendages on the surface of the chasmothecia help the fungi adhere to host plant surfaces. Appendages can also be used as morphological features to aid in the identification of powdery mildew fungi (Glawe, 2008).

Occurrence of cucurbit powdery mildew in Illinois

In Illinois, powdery mildew on cucurbits develops around the middle of July and is commonly observed in August and September in commercial production fields. Powdery mildew causes yield losses of more than 50% in Illinois (Babadoost, 2016; Babadoost et al., 2017). The primary sources of initial inoculum in Illinois and other Midwestern states have not been determined. However, it is likely that airborne conidia travel over long distances from the southern states of US, where cucurbits are gown in winter or planted earlier in the season. In addition, inoculum may also come from infected cucurbits in local greenhouses. As obligate parasites, powdery mildew fungi can only survive on living host plant tissue and as overwintering chasmothecia. Chasmothecia are rarely found in fields in the US (McGrath et al., 1996) and have not been reported on cucurbits in Illinois.

Identification of powdery mildew fungi

Like most fungi, identification of the powdery mildew fungi on cucurbits mainly depends on the morphological features of the fruiting structures and nucleic acid sequence information. Morphological identification of powdery mildew fungi on cucurbits is based mainly on the features of asexual stages, partly due to lack of chasmothecia. Characteristics used for identifying species include the shape and size of conidia, the presence of fibrosin bodies, and the morphology of the germ tubes (Křístková et al., 2009; Lebeda, 1983). The molecular methods used for identification of species are based on the sequences of the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA (nrDNA) (Al-Sadi et al., 2012; Chen et al., 2008; Garibaldi et al., 2010; Perez-Garcia et al., 2009; Ramos et al., 2010).

Intensive research on the identification of powdery mildew fungi on cucurbits began in 1979 and has continued. From 1979 to 1980, 102 powdery mildew samples from cucurbits collected in 37 locations in Czechoslovakia were identified as *E. cichoracearum* and *S. fuliginea* based on the morphology of their imperfect conidial stage. Meanwhile, the investigators found *E. cichoracearum* predominated in open fields while *S. fuliginea* was more common in glasshouses (Lebeda, 1983). In 1994, 275 infected cucurbit leaf samples collected in five regions in France were identified as *E. cichoracearum* and *S. fuliginea*, at levels of 20 and 80%, respectively, based on the morphology of conidia and conidiophores (Bardin et al., 1999). From 1995 to 2000, *P. xanthii* and *G. cichoracearum* were determined as the species causing powdery mildew on cucurbits in Czechoslovakia, some other European countries, and the Middle East. The species were identified by analyzing the morphological characteristics of the rehydrated dry conidia. *P. xanthii* was the only powdery mildew species found in Israel, Spain, and Turkey; *P. xanthii* and *G. cichoracearum* were found in Austria, France, Germany, Great Britain, Italy, Slovakia, Slovenia, Switzerland, and the Netherlands; *G. cichoracearum* was found to be the dominant (up to 98.8%) species in Czechoslovakia, which is located in the more northerly latitudes and higher elevations (Křístková et al., 2009). From 2002 to 2006, 82 cucurbits powdery mildew isolates from 34 fields in southern Italy were all identified as *P. xanthii* based on the morphological features of conidia and germ tubes and the presence of fibrosin bodies (Miazzi et al., 2011). In 2011, *P. xanthii* was identified by molecular methods as the only species causing powdery mildew on cucurbits in a greenhouse in Salinas, California. Molecular identification was done using polymerase chain reaction (PCR) and S1/S2 primers (Chen et al, 2008). To our knowledge, there is no published information on the identification of powdery mildew fungi on cucurbits in Illinois or the Midwestern states of the US.

Previously, identification of powdery mildew fungi on most other hosts was mostly based on morphological features of the chasmothecia. Now, morphological identification of powdery mildew fungi requires both sexual and asexual stage attributes (Heffer, 2006) and molecular methods. The incorporation of DNA sequencing data has greatly revised powdery mildew fungal taxonomy.

Pathogenicity variation

Pathogenicity refers to a qualitative trait of genetic capability of a microorganism to cause disease (Sacristan and Garcia-Arenal, 2008). Breeding cucurbit cultivars that are resistant to powdery mildew greatly depends on knowledge of the pathogenicity of powdery mildew fungi in a production area. Some reports on rating the pathogenicity of isolates of powdery mildew fungi have been published. However, studies regarding the pathogenic variation of powdery mildew fungi on cucurbits have received limited attention. Of 41 *E. cichoracearum* isolates studied, 90%, 56%, and 2% of the isolates were pathogenic to cucumber cultivar Marketer, zucchini cultivar Diamant, and watermelon cultivar Sugar Baby, respectively (Bardin et al., 1999).

Genetic variation within species of powdery mildew fungi

Information on genetic variation helps us to better understand the evolutionary process and genetic linkage, in order to develop effective management strategies of plant diseases (Burdon, 1993). Plant pathologists adopted the use of molecular markers to investigate genetic variation in pathogen populations. Molecular markers have been used to test hypotheses based on genetic variations within a certain population of powdery mildew fungi, such as the variations

occurring in space and time, or the interactions between pathogen and host genotypes (Al-Sadi et al., 2012; Bardin et al., 1999; Miazzi et al., 2011). About two decades ago, genetic variation of the powdery mildew pathogen *Erysiphe graminis* f.sp. *hordei* from barley was investigated in order to understand the evolutionary processes and genetic linkage in conjunction with virulence and fungicide sensitivity. Random amplified polymorphic DNA (RAPD) markers were developed to detect variations such as multiple alleles, or the presence and absence of specific squences of DNA (McDermott et al., 1994). To determine the relationships between the genetic variation and the origins of inoculum, Núñez et al. (2006a) examined 31 *Erysiphe necator* isolates from grapes in Spain with 61 amplified fragment length polymorphism (AFLP) markers that separated into two distinct groups with 63% genetic similarity. The results did not show any significant correlation between original inocula (overwintering ascomata or mycelia) and genetic variation among isolates of the fungus. In order to determine if the genetic variation was associated with geographical origins and host, 41 *E. cichoracearum* isolates from cucurbits were assessed based on 147 RAPD fragments generated using 16 primers. The isolates were divided into three groups with distinct rDNA haplotypes. It was found that the grouping of genetic differentiations was not strongly correlated with geographical origin, but the results indicated a possible host-specialized subdivision within the population (Bardin et al., 1999). Another study on the powdery mildew pathogen *Blumeria graminis* from cereals resulted in a slightly different conclusion. Analysis of nuclear gene regions divided isolates of *B. graminis* into two clades, which correlated with both host pathogenicity and geographic area (Troch et al., 2012). The genetic variations of powdery mildew pathogens from cucurbits in Illinois and their relationship with geographic origins, host, and variations in pathogenicity have not been investigated.

Clustering methods

There are two clustering methods commonly applied to genomic data for genetic variation analysis: hierarchical clustering and non-hierarchical clustering. Hierarchical clustering starts with N clusters (N = the sample number) where each cluster contains exactly one sample. Based on the Euclidean distance, two closest points are combined as one cluster and the combination goes on until a single cluster is reached. The K-means clustering algorithm takes the matrix with N samples and a given partition number K, initialized with K-cluster centers with the mean of each cluster $\mu_1, \ldots, \mu_K$. Through iteration, each sample is re-assigned to the closest initial cluster center and the new cluster center μ_K is recomputed; this process is repeated until the minimum within-cluster variation is reached for the assigned K cluster. Euclidean distance method is also commonly used for the distance calculation for K-means clustering (James et al. 2013).

As one of the most commonly used statistics in population genetics, fixation index (Fst) is a measure of population differentiation due to genetic structure in genetic polymorphism data such as single nucleotide polymorphism (SNPs) (Holsinger et al., 2017).

Fungicide management of powdery mildew on cucurbits

Management of powdery mildew on cucurbits has been heavily dependent on using resistant cultivars and the application of fungicides. However, the resistance cultivars have been constantly overcome by more virulent pathogen strains (McGrath, 2001). Although the use of fungicides is essential for management of powdery mildew on cucurbits, resistance development in pathogens to fungicides is a serious problem (Bellón-Gómez et al., 2014; McGrath, 2001). Powdery mildew on cucurbits has been managed using several fungicides with different modes

of actions. The fungicides available for the management of powdery mildew of cucurbits in Illinois and other Midwestern states are demethylase inhibitors (DMI); Quinone outside Inhibitors (QoI); succinate dehydrogenase inhibitor (SDHI); quinolones; and cyflufenamid, a new fungicide with a mode of action yet to be determined.

DMI fungicides are the most commonly used fungicides in agriculture. There are more than 30 fungicides in this single-site mode of action group, including imidazoles, piperazines, pyridines, pyrimidines, and triazoles. This group targets the site of C14-demethylase in sterol biosynthesis, depletes the major fungal membrane component ergosterol, accumulates potentially toxic sterol intermediates, and eventually changes membrane structure and function (Lóopez-Ruiz et al., 2010). DMI fungicides have remained highly effective despite a long history of intensive use (Babadoost et al., 2017). However, like many other plant pathogenic fungi, powdery mildew fungi on cucurbits have been reported to have developed resistance to DMI fungicides. The reported cases include resistance to myclobutanil and triflumizole in North Carolina (Adams et al., 2007), triadimefon in New York (McGrath, 2001), and fenarimol and triadimenol in Spain (Lóopez-Ruiz et al., 2010). In Illinois, the use of DMI fungicides for cucurbit powdery mildew treatment first began when these fungicides became available. The DMI fungicide triflumizole, formulated as Procure 480SC has been used in Illinois since it was registered in 2001.

QoI fungicides are very important because of their high effectiveness towards many plant diseases and low-risk to the environment. This single-site mode of action fungicide group includes strobilurins and some other inhibitors of the Qo site of the cytochrome bc1 complex (McGrath, 2001). Resistance of powdery mildew fungi to QoI fungicides has developed quickly due to the intensive use of these fungicides. In cucumber production areas in Japan, *P. fusca*

strains resistant to the strobilurin fungicides azoxystrobin and kresoxim-methyl were found in 1998 and 1999 (Ishii et al., 2001). In 2002, *P. xanthii* isolates collected from research sites in Georgia, New York and North Carolina were found to be resistant to the strobilurin fungicides trifloxystrobin, formulated as Flint, and azoxystrobin, formulated as Quadris. This was the first report of finding *P. xanthii* isolates with resistance to strobilurins in the US (McGrath and Shishkoff, 2003). In cucurbit production areas in Spain, *P. fusca* isolates collected from 2002 to 2004 were found to be resistant to azoxystrobin, kresoxim-methyl, and trifloxystrobin (Fernandez-Ortuno et al., 2006; Fernández-Ortuño et al., 2008). As one of the strobilurin fungicides, azoxystrobin was registered in the US in 1998.

SDHI fungicides were discovered more than 40 years ago. This single-site mode of action fungicide group includes several carboxamides and some other inhibitors targeting the enzyme succinate dehydrogenase. This enzyme plays an important role in the tricarboxylic acid cycle and the mitochondrial electron transport chain (Miyamoto et al., 2010). As a group of fungicides with medium to high level of risk, powdery mildew isolates from cucurbits with resistance have been reported. *P. xanthii* isolates collected from cucumbers from the commercial greenhouses in Japan were reported to have reduced sensitivities to boscalid, a commonly used SDHI (Miyamoto et al., 2010). Another SDHI fungicide, penthiopyrad was formulated as Fontelis 1.67SC and introduced to the US in 2012. It has been used in Illinois since then.

Quinoxyfen and cyflufenamid are two commonly used fungicides for the management of powdery mildew on cucurbits in Illinois (Babadoost, 2010; Babadoost et al., 2017). Proposed to target signal transduction, quinexyfen, a member of the quinolines fungicide group, is a protectant/systematic fungicide inhibiting the early stages of mildew infection (Hollomon et al., 1997). Quinoxyfen is suggested as a fungicide with low risk for resistance development for two

reasons. First, lab tests have shown that mutant isolates of powdery mildew species *Erysiphe graminis* f.sp. *hordei* from barley have low fitness with reduced conidia production. Second, quinoxyfen is usually recommended for only one single early treatment (Hollomon et al., 1997). Although no conclusive results have been reported for resistance to quinoxyfen among powdery mildew isolates from cucurbits in the US, variation in sensitivities of *P. xanthii* isolates from pumpkins to quinoxyfen suggested that the potential risk of resistance development in the pathogen population to this fungicide over time (Davey and McGrath, 2006). Quintec 250SC is a fungicide with active ingredient quinexyfen, which is extensively used for managing powdery mildew of cucurbits. It has been used in Illinois for management of powdery mildew of cucurbits since it was registered (Babadoost, 2010; Babadoost et al., 2017).

Modes of actions of cyflufenamid are yet to be determined. Cyflufenamid is formulated as Torino 0.85SC. Little is known about the biochemical mechanisms of cyflufenamid on powdery mildew fungi. Resistance to cyflufenamid has been reported in *P. xanthii* isolates from melon and zucchini in Italy in 2012 and 2013 (Pirondi et al., 2015b).

Cucurbit powdery mildew fungi have been treated with fungicides from all five of the fungicide groups mentioned above. The detection of any variation in sensitivity among isolates associated with resistance to other fungicide groups is valuable (Davey and McGrath, 2006).

Objectives of this study were:

Objective 1. Identification of fungal species causing powdery mildew on cucurbits in Illinois and some other major cucurbits production areas

Objective 2. Determine genetic and pathogenic variations among isolates of powdery mildew fungi on cucurbits

Objective 3. Determine the sensitivities of cucurbit powdery mildew fungi to major powdery mildew fungicides

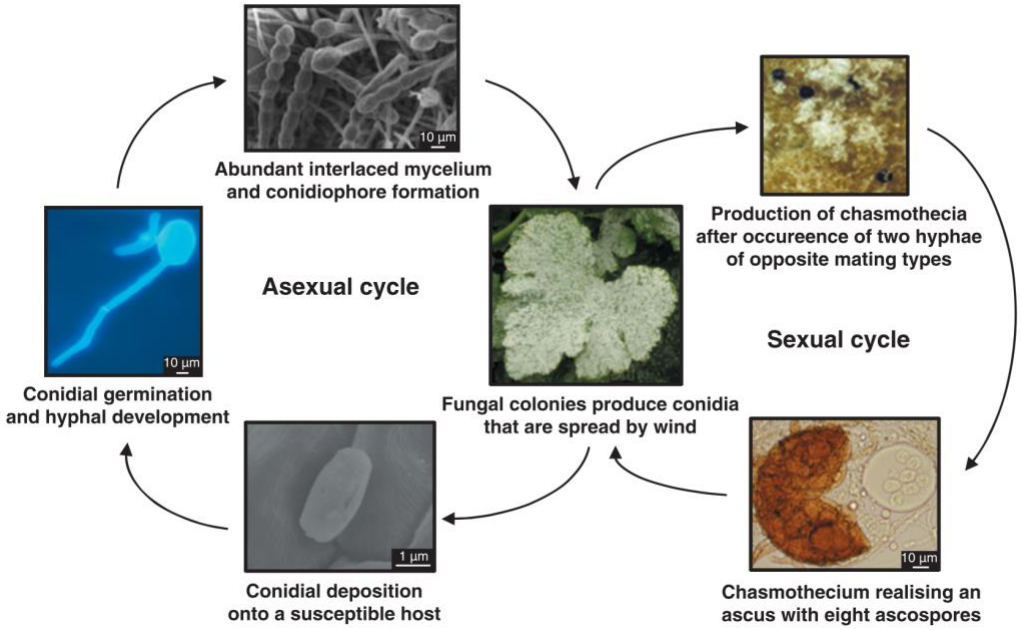

Figure 1.1. The life cycle of powdery mildew disease of cucurbits (Glawe, 2008)

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CHAPTER 2
IDENTIFICATION OF FUNGAL SPECIES CAUSING POWDERY MILDEW ON CUCURBITS IN ILLINOIS AND SOME OTHER MAJOR CUCURBIT PRODUCTION AREAS

ABSTRACT

This study was conducted to identify species of fungi causing powdery mildew on cucurbits. During the period of 2014-2016, 201 leaf samples of cucumbers, gourds, cantaloupe, jack-o-lantern pumpkins, processing pumpkins, and summer and winter squashes were collected from California, Illinois, Indiana, Iowa, Michigan, New York, Pennsylvania, Texas, Washington, and Wisconsin. Pure colonies of the causal fungi were prepared and the morphology of conidia of the isolates was studied. The internal transcribed spacer (ITS) region of nuclear ribosomal DNA (nrDNA) from 119 of the 201 isolates was sequenced. ITS sequences of these isolates were compared to existing sequences in GenBank, and these comparisons revealed that all of the isolates belonged to the species *Podosphaera xanthii*.

INTRODUCTION

morphological features of the fruiting structures and nucleic acid sequence information are two main approaches for identification of the powdery mildew fungi on cucurbits. Due to lack of chasmothecia, the morphological identification of powdery mildew fungi on cucurbits is based mainly on the features of asexual stages. Characteristics used for identifying species include the shape and size of conidia, the presence of fibrosin bodies, and the morphology of the germ tubes (Křístková et al., 2009; Lebeda, 1983). Based on the sequences of the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA (nrDNA), the species can be

identified using the molecular methods (Al-Sadi et al., 2012; Chen et al., 2008; Garibaldi et al., 2010; Perez-Garcia et al., 2009; Ramos et al., 2010).

MATERIALS AND METHODS

Sample collections

From July-September 2014, 2015, and 2016, leaf samples of cucurbits with symptoms of powdery mildew were collected from California (CA), Illinois (IL), Indiana (IN), Iowa (IA), Michigan (MI), Pennsylvania (PA), Texas (TX), Washington (WA), and Wisconsin (WI). In addition, 10 DNA samples from Italy and 40 isolates from New York and Wisconsin of cucurbit powdery mildew fungi were obtained (Figure 4.1). The infected leaf samples from Illinois were collected from 13 counties (Figure 4.2). In addition, cultures of cucurbit powdery mildew fungi were provided from New York (Margaret McGrath, Cornell University); and DNA of isolates of cucurbit powdery mildew fungi were provided from Italy and Chile (Alessandro Pirondi, Universita degli Studi di Bologna in Italy) (Table 2.1).)

The samples were collected from commercial fields of cantaloupe (*Cucumis melo*), cucumber (*Cucumis sativus*), Jack-o-lantern pumpkin (*Cucurbita pepo*), processing pumpkin (*Cucurbita moschata*), watermelon (*Citrullus lanatus*), winter squash (*Cucurbita maxima*), yellow squash (*Cucurbita pepo*), and zucchini (*Cucurbita pepo*).

Isolation of samples

Pure colonies were prepared from each sample as suggested by Nicot et al. (2002). Seeds of cucumber 'Bush Crop' (Rupp Seeds Inc., Wauseon, Ohio, USA), a susceptible cultivar to powdery mildew, were sown in Sunshine Mix LC1 (Sun Gro Horticulture, Agawam, MA) in 27

× 54 × 6 cm flats at a rate of 90 seeds per flat. Plants were grown in a greenhouse at 22.5 - 25°C during daytime and 16.5 - 18.5°C at night, with 14 h photoperiod at a light intensity of 436 $\mu E/m^2/s$. Plants were watered daily.

Approximately one week after sowing seeds, fully expanded cotyledon leaves developed and were harvested. The cotyledon leaves were rinsed with tap water to remove soil particles, rinsed with 0.6% sodium hypochlorite, followed by rinsing twice with sterile distilled water (SDW) to disinfest the surfaces of the leaves. Three clean cotyledon leaves were placed in a 90 mm-diameter Petri dishes containing 20 ml water agar with antibiotic (WAA) (18 g agar, 20 mg pimaricin in 1000 ml distilled water). All cotyledon leaves in the Petri dishes were placed with their abaxial surfaces contacting the media. Plates with cotyledons were left in a laminar flow hood for 1 hours to air dry before inoculation.

For each collected leaf sample with powdery mildew, two colonies were prepared. Conidia from these samples were transferred onto separate surface-disinfested cotyledon leaves on WAA using sterilized pipette tips. Single-spore colonies of 119 samples were prepared for this study (Table 2.2). The inoculated cotyledon leaves were placed in a growth chamber at 25°C with 12 h light/12 h dark for two weeks before DNA extraction. The cultures were maintained by sub-culturing every two weeks on detached and surface-disinfested cucumber cotyledons on WAA in Petri dishes. Species of the powdery mildew fungi were identified using both the morphological characteristics of conidia and sequencing DNA extracted from mycelia and conidia.

Morphological characters

Thirty-two isolates of the powdery mildew fungi collected in 2014 and 2015 were randomly selected and the morphological characteristics of their conidia were studied using a compound light microscope (Optical Analysis Corporation, Nashua, NH, USA). The following morphological characteristics were recorded: 1) lengths and widths of 25 arbitrarily chosen conidia from each isolate, and their minimum, maximum, mean and standard deviation were calculated; 2) the presence of fibrosin bodies associated with the conidia, which is considered the major morphological difference between *Podosphaera xanthii* (syn.: *Sphaerotheca fuliginea*) and *Golovinomyces cichoracearum* (Syn.: *Erysiphe cichoracearum*).

Molecular identification

For molecular identification, 119 isolates from various cucurbit hosts and from numerous areas throughout the USA (Table 2.2) were processed. Mycelia and conidia from each isolate were scraped from inoculated cotyledons and DNA was extracted using the E.Z.N.A MicroElute Genomic DNA kit (Omega Bio-Tek Inc; Norcross, Georgia, USA). Polymerase chain reaction (PCR) was performed for the entire internal transcribed spacer (ITS) region from nuclear ribosomal DNA (nrDNA), using fungus-specific primers ITS1F (5' - CTT GGT CAT TTA GAG GAA GTA A-3') and ITS4 (5' - GCA TAT CAA TAA GCG GAG GA-3') (Gardes and Bruns, 1993; White et al., 1990). PCR amplification was conducted using GE Healthcare Illustra PuReTag Ready-To-Go PCR beads in 25 µL reactions containing 3 µL DNA, 15 µL nuclease-free distilled water, 2.5 µL 50% DMSO, 2.5 µL bovine serum albumin (BSA), 1 µL of 10 µM forward primer ITS1F, and 1 µL of 10 µM reverse primer ITS4. PCR was run under the following conditions: 95°C for 5 minutes, followed by 39 cycles of 95°C for 30 sec, 43°C for 15

sec, and 72°C for 1 min, with a final extension at 72°C for 10 min. The PCR products were purified using a Promega Wizard SV Gel and PCR Clean up kit (Promega; Madison, Wisconsin, USA). The cleaned PCR products were sequenced in both directions using the same ITS1F/ITS4 primers. Contigs were assembled automatically, checked by eye, and both primers were inserted into each contig using Sequencher 5.1 (Gene Code Corporation, Ann Arbor, MI, USA) before creating consensus sequences. Consensus sequences from each isolate were aligned and searched using the basic local alignment search tool (BLAST) at the National Center for Biotechnology Information (NCBI) (www.ncbi.nlm.nih.gov/BLAST) to determine fungal identification.

RESULTS

Morphological characteristics

After examining 25 arbitrarily chosen conidia from each isolate, it was found that the length of conidia from 32 isolates ranged from 9.00 to 50.00 μm, and their mean length was 30.01 μm and standard deviation was 7.03; the width of conidia from 32 isolates ranged from 6.00 to 35.00 μm, and their mean length was 18.43 μm and standard deviation was 4.09 (Table 2.3). The conidia were barrel-shaped, and the fibrosin bodies were observed among the conidia, and the angular shapes at connection points of conidia in chains were found in all isolates studied. These morphological characteristics match the description of *P. xanthii* (Braun and Cook, 2012). No chasmothecia were found in any of the collected leaf samples.

Molecular identification

Sequences from all 119 isolates, including collections from US and Italy, were 601 bp, which had 100% identity with GenBank accession JQ728480, a *P. xanthii* isolate found on *Senna occidentalis* in Mexico. The sequence variability among the 119 isolates was minimum.

DISCUSSION

Molecular analysis of the ITS region of powdery mildew causing fungi has been thoroughly studied, and considerable data have been deposited in GenBank. We sequenced the ITS regions of 119 isolates collected from Illinois and other cucurbit growing areas in the USA and Italy, and the results were compared with the data in GenBank. The comparison showed 100% match to *P. xanthii*.

The ITS region was determined to be homogeneous for all of the *P. xanthii* isolates collected from different cucurbit species and across all of the collected geographical origins. Morphological characteristics of conidia also supported the identification of the isolates as *P. xanthii*, which were collected from six cucurbit species in CA, IL, IN, MI, NY, TX, WA, and WI in the USA. During our studies, no chasmothecia of the powdery mildew fungus were found either in the surveyed commercial fields or in the laboratory studies. Similar findings were reported by Perez-Garcia et al. (2009). Chasmothecia are considered major overwintering structures of powdery mildew fungi in the absence of host plants. Considering that no cucurbits are grown in Illinois during the winter, additional studies will be needed to determine primary inoculum sources of powdery mildew fungi in Illinois and other states in North Central Region.

Table 2.1. Host species and their locations of cucurbit powdery mildew fungi used for molecular identification of causal species.

	Number of powdery mildew samples from each location[z]									Total sample no.
Plant host	**CA**	**IL**	**IN**	**MI**	**NY**	**TX**	**WA**	**WI**	**Italy**	
Cucurbita maxima	3	5		3						11
Cucumis melo		4							2	6
Cucurbita moschata		8		2						10
Cucurbita pepo	10	40	3	5	5	5	5	2	1	76
Cucumis sativus		6				4			1	11
Lagenaria siceraria							1			1
Unknown	4									4
Total	17	63	3	10	5	9	6	2	4	119

[z] CA = California, IL = Illinois, IN = Indiana, MI = Michigan, NY = New York, TX = Texas, WA = Washington, and WI = Wisconsin.

Table 2.2. Year of collection, host, and location of the sample for 119 isolates of powdery mildew fungi used in for molecular identification.

			Location of collected samples	
Isolate	**Year**	**Host Plant**	**State[z]**	**Country[y]**
CA9_1	2016	*Cucurbita maxima*	CA	US
CA9_2	2016	*Cucurbita maxima*	CA	US
CA9_3	2016	*Cucurbita maxima*	CA	US
CA1_2	2016	*Cucurbita pepo*	CA	US
CA1_3	2016	*Cucurbita pepo*	CA	US
CA2_1	2016	*Cucurbita pepo*	CA	US
CA2_2	2016	*Cucurbita pepo*	CA	US
CA2_3	2016	*Cucurbita pepo*	CA	US
CA5_1	2016	*Cucurbita pepo*	CA	US
CA5_2	2016	*Cucurbita pepo*	CA	US
CA5_3	2016	*Cucurbita pepo*	CA	US
CA6_1	2016	*Cucurbita pepo*	CA	US
CA6_2	2016	*Cucurbita pepo*	CA	US
CA4_1	2016	Unknown	CA	US
CA4_2	2016	Unknown	CA	US
CA5+9	2016	Unknown	CA	US
CA8_1	2016	Unknown	CA	US
IL39_ZGH	2014	*Cucurbita pepo*	IL	US
IL05_Q5	2014	*Cucumis sativus*	IL	US
IL37_Q7	2014	*Cucumis sativus*	IL	US
IL38_Q8	2014	*Cucumis sativus*	IL	US
IL04_CL0829_3	2015	*Cucumis melo*	IL	US
IL25_WS082302_1	2015	*Cucurbita maxima*	IL	US
IL26_WS082302_3	2015	*Cucurbita maxima*	IL	US
IL27_WS082304_2	2015	*Cucurbita maxima*	IL	US
IL20_PP082401_2	2015	*Cucurbita moschata*	IL	US
IL21_PP082404_1	2015	*Cucurbita moschata*	IL	US
IL2300	2015	*Cucurbita moschata*	IL	US
IL01_0701_E_E	2015	*Cucurbita pepo*	IL	US
IL02_0701L	2015	*Cucurbita pepo*	IL	US
IL03_0701_E_T2	2015	*Cucurbita pepo*	IL	US
IL07_JP0809_1	2015	*Cucurbita pepo*	IL	US
IL08_JP0809_2	2015	*Cucurbita pepo*	IL	US
IL10_JP0816_4	2015	*Cucurbita pepo*	IL	US
IL11_JP081601_2	2015	*Cucurbita pepo*	IL	US
IL12_JP081602_2	2015	*Cucurbita pepo*	IL	US
IL13_JP081602_3	2015	*Cucurbita pepo*	IL	US
IL24_P08230202	2015	*Cucurbita pepo*	IL	US

Table 2.2 (cont.)

IL28_YS0816_3	2015	*Cucurbita pepo*	IL	US
IL29_YS082303_1	2015	*Cucurbita pepo*	IL	US
IL30_YS0829_1	2015	*Cucurbita pepo*	IL	US
IL31_YS0829_3	2015	*Cucurbita pepo*	IL	US
IL33_YS0725_2	2015	*Cucurbita pepo*	IL	US
IL34_Z0816_2	2015	*Cucurbita pepo*	IL	US
IL35_JP0809_5	2015	*Cucurbita pepo*	IL	US
IL36_YS082303_2	2015	*Cucurbita pepo*	IL	US
IL14_JP081603_3	2015	*Cucurbita pepo*	IL	US
IL15_JP082301_2	2015	*Cucurbita pepo*	IL	US
IL18_NP0906_2	2015	*Cucurbita pepo*	IL	US
IL19_NP0906_3	2015	*Cucurbita pepo*	IL	US
IL16_JPGH0829	2015	*Cucumis sativus*	IL	US
IL17_JPGH	2015	*Cucumis sativus*	IL	US
ILD1_16	2016	*Cucumis melo*	IL	US
ILD2_16	2016	*Cucumis melo*	IL	US
ILD3_16	2016	*Cucumis melo*	IL	US
IL3_16	2016	*Cucurbita maxima*	IL	US
IL3_3_16	2016	*Cucurbita maxima*	IL	US
IL1_16	2016	*Cucurbita moschata*	IL	US
IL1_3_16	2016	*Cucurbita moschata*	IL	US
ILN3_2_16	2016	*Cucurbita moschata*	IL	US
ILV1_1_16	2016	*Cucurbita moschata*	IL	US
ILV1_3_16	2016	*Cucurbita moschata*	IL	US
IL2_2_16	2016	*Cucurbita pepo*	IL	US
IL2_4_16	2016	*Cucurbita pepo*	IL	US
IL4_2_16	2016	*Cucurbita pepo*	IL	US
IL5_16	2016	*Cucurbita pepo*	IL	US
IL7_16	2016	*Cucurbita pepo*	IL	US
IL7_2_16	2016	*Cucurbita pepo*	IL	US
ILC1_16	2016	*Cucurbita pepo*	IL	US
ILC2_16	2016	*Cucurbita pepo*	IL	US
ILC3_2_16	2016	*Cucurbita pepo*	IL	US
ILII_1_16	2016	*Cucurbita pepo*	IL	US
ILII_2_16	2016	*Cucurbita pepo*	IL	US
ILV3_3_16	2016	*Cucurbita pepo*	IL	US
ILV3_4_16	2016	*Cucurbita pepo*	IL	US
ILV4_1_16	2016	*Cucurbita pepo*	IL	US
ILV4_3_16	2016	*cucurbita pepo*	IL	US
ILVC2_16	2016	*Cucurbita pepo*	IL	US
ILVC3_16	2016	*Cucurbita pepo*	IL	US
ILGHQ16_16	2016	*Cucumis sativus*	IL	US

Table 2.2 (cont.)

IN3	2016	*Cucurbita pepo*	IN	US
IN3_2	2016	*Cucurbita pepo*	IN	US
IN4	2016	*Cucurbita pepo*	IN	US
MI2_1	2016	*Cucurbita maxima*	MI	US
MI2_2	2016	*Cucurbita maxima*	MI	US
MI5_1	2016	*Cucurbita maxima*	MI	US
MI4_1	2016	*Cucurbita moschata*	MI	US
MI4_2	2016	*Cucurbita moschata*	MI	US
MI1_1	2016	*Cucurbita pepo*	MI	US
MI3-1	2016	*Cucurbita pepo*	MI	US
MI6_1	2016	*Cucurbita pepo*	MI	US
MI6_2	2016	*Cucurbita pepo*	MI	US
MI7_2	2016	*Cucurbita pepo*	MI	US
NY-11-2F	2015	*Cucurbita pepo*	NY	US
NY-11-5A	2015	*Cucurbita pepo*	NY	US
NY32_11_1F	2015	*Cucurbita pepo*	NY	US
NY33_11_5F	2015	*Cucurbita pepo*	NY	US
NY34_S9	2015	*Cucurbita pepo*	NY	US
TX1	2016	*Cucurbita pepo*	TX	US
TX1_1	2016	*Cucurbita pepo*	TX	US
TX1_2	2016	*Cucurbita pepo*	TX	US
TX1_3	2016	*Cucurbita pepo*	TX	US
TX1_4	2016	*Cucurbita pepo*	TX	US
TX3	2016	*Cucumis sativus*	TX	US
TX3_2	2016	*Cucumis sativus*	TX	US
TX3_3	2016	*Cucumis sativus*	TX	US
TX3_4	2016	*Cucumis sativus*	TX	US
WA1_1	2016	*Cucurbita pepo*	WA	US
WA1_2	2016	*Cucurbita pepo*	WA	US
WA1_3	2016	*Cucurbita pepo*	WA	US
WA1_4	2016	*Cucurbita pepo*	WA	US
WA1_5	2016	*Cucurbita pepo*	WA	US
WA2	2016	*Lagenaria siceraria*	WA	US
WI3_2	2016	*Cucurbita pepo*	WI	US
WI4_3	2016	*Cucurbita pepo*	WI	US
Italy7_5138	2015	*Cucumis melo*	Mantova	Italy
Italy6_23136	2015	*Cucurbita pepo*	Mantova	Italy
Italy8_CMS13cb	2015	*Cucumis melo*	Modena	Italy
Italy10_CSC13c	2015	*Cucumis sativus*	Modena	Italy

[z] CA = California, IL = Illinois, IN = Indiana, MI = Michigan, NY = New York, TX = Texas, WA = Washington, and WI = Wisconsin.

[y] US = United States.

Table 2.3. Dimensions of conidia of isolates of powdery mildew fungi collected from Illinois.

				Dimensions of conidia (µm)			
Isolates	**Year**	**Host plant**	**County[z]**	**Length**	**Std[y]**	**Width**	**Std**
IL17_JPGH	2014	*Cucurbita pepo*	Champaign	34.32	5.56	19.88	1.74
IL06_QGH	2014	*Cucumis sativus*	Champaign	31.96	2.32	18.44	1.39
IL37_Q7	2014	*Cucumis sativus*	Champaign	33.24	3.03	18.56	1.61
IL38_Q8	2014	*Cucumis sativus*	Champaign	31.84	7.40	20.24	1.98
IL05_Q5	2014	*Cucumis sativus*	Kane	34.56	3.25	20.00	2.99
IL04_CL0829_3	2015	*Cucumis melo*	Champaign	33.56	2.92	20.52	2.00
WS082301	2015	*Cucurbita maxima*	Kane	32.32	3.65	18.68	1.97
WS082303	2015	*Cucurbita maxima*	Kane	33.96	4.41	17.96	2.73
PP0829	2015	*Cucurbita moschata*	Champaign	32.08	4.38	19.76	1.94
IL20_PP082401_2	2015	*Cucurbita moschata*	Tazewell	32.04	4.06	20.22	1.78
IL21_PP082404_1	2015	*Cucurbita moschata*	Tazewell	28.58	1.35	20.21	0.72
PP082402	2015	*Cucurbita moschata*	Tazewell	33.91	4.09	20.48	1.20
PP082403	2015	*Cucurbita moschata*	Tazewell	32.00	2.49	19.91	1.28
IL11_JP081601_2	2015	*Cucurbita pepo*	Calhoun	29.04	1.79	19.56	1.16
IL31_YS0829_3	2015	*Cucurbita pepo*	Champaign	32.04	3.47	19.40	2.00
IL01_0701_E_E	2015	*Cucurbita pepo*	Champaign	32.48	2.76	18.92	1.55
IL02_0701L	2015	*Cucurbita pepo*	Champaign	33.88	3.10	18.68	2.39
IL39_ZGH	2015	*Cucurbita pepo*	Champaign	33.60	4.38	21.56	2.84
JP0829_2	2015	*Cucurbita pepo*	Champaign	32.72	3.90	20.96	1.88
IL03_0701-E-T2	2015	*Cucurbita pepo*	Champaign	31.76	2.93	18.80	2.12
YS0731	2015	*Cucurbita pepo*	Champaign	19.88	0.88	19.88	0.88
Z0829	2015	*Cucurbita pepo*	Champaign	32.16	6.93	20.16	3.39
IL12_JP081602_2	2015	*Cucurbita pepo*	Douglas	27.68	2.14	20.20	0.82
P083101	2015	*Cucurbita pepo*	Douglas	30.83	2.82	18.50	2.36
Z0725	2015	*Cucurbita pepo*	Douglas	30.12	4.07	20.80	2.78
JP083101	2015	*Cucurbita pepo*	Fayette	14.42	5.31	8.42	0.83
JP083102	2015	*Cucurbita pepo*	Fayette	12.79	1.47	10.42	14.41
IL24_P08230202	2015	*Cucurbita pepo*	Kane	34.71	2.80	19.75	1.11
YS082401	2015	*Cucurbita pepo*	Marshall	32.42	4.34	19.38	1.69
Z082401	2015	*Cucurbita pepo*	Marshall	31.96	3.26	20.58	1.82
IL29_YS082303_1	2015	*Cucurbita pepo*	McHenry	32.12	2.47	19.68	1.63
IL07_JP0809_1	2015	*Cucurbita pepo*	McHenry	28.68	6.28	18.32	1.99

[z] Counties in Illinois.
[y] Standard deviation.

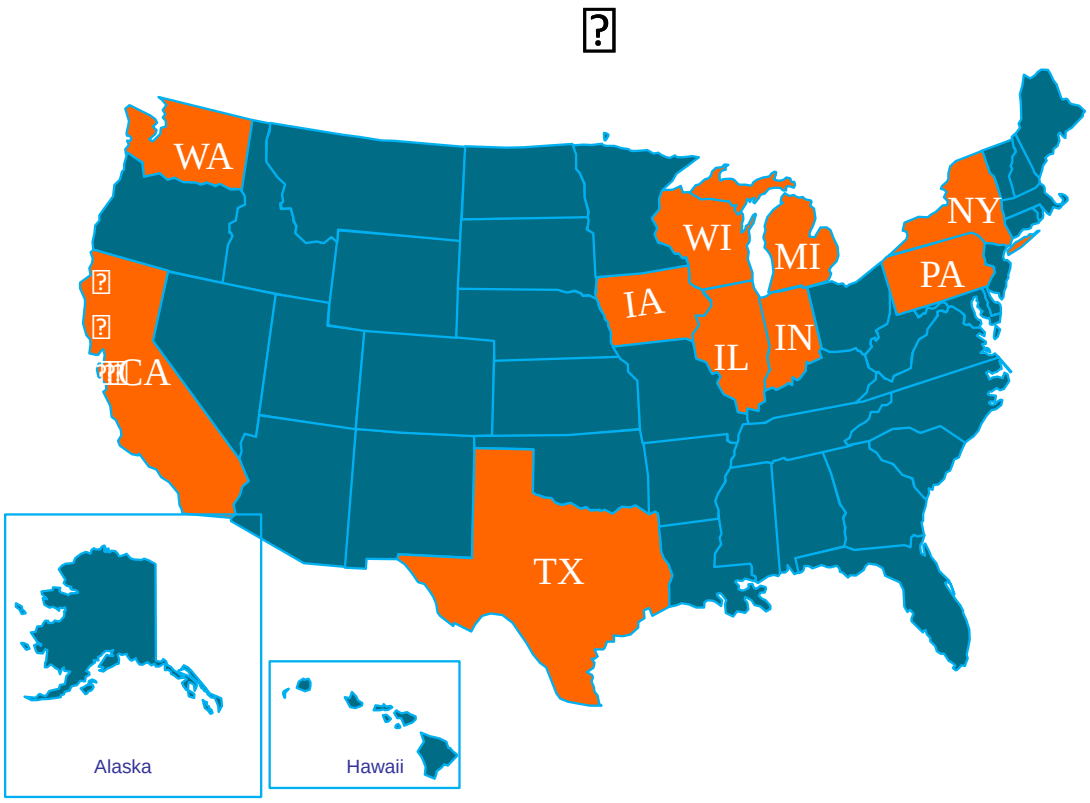

Figure 2.1. States (in orange) in the United States (in orange) where leaves of cucurbit crops with powdery mildew were collected in 2014–2016. CA = California; IA = Iowa; IL = Illinois; IN = Indiana; MI = Michigan; NY=New York; PA=Pennsylvania; TX = Texas; WA=Washington, and WI=Wisconsin.

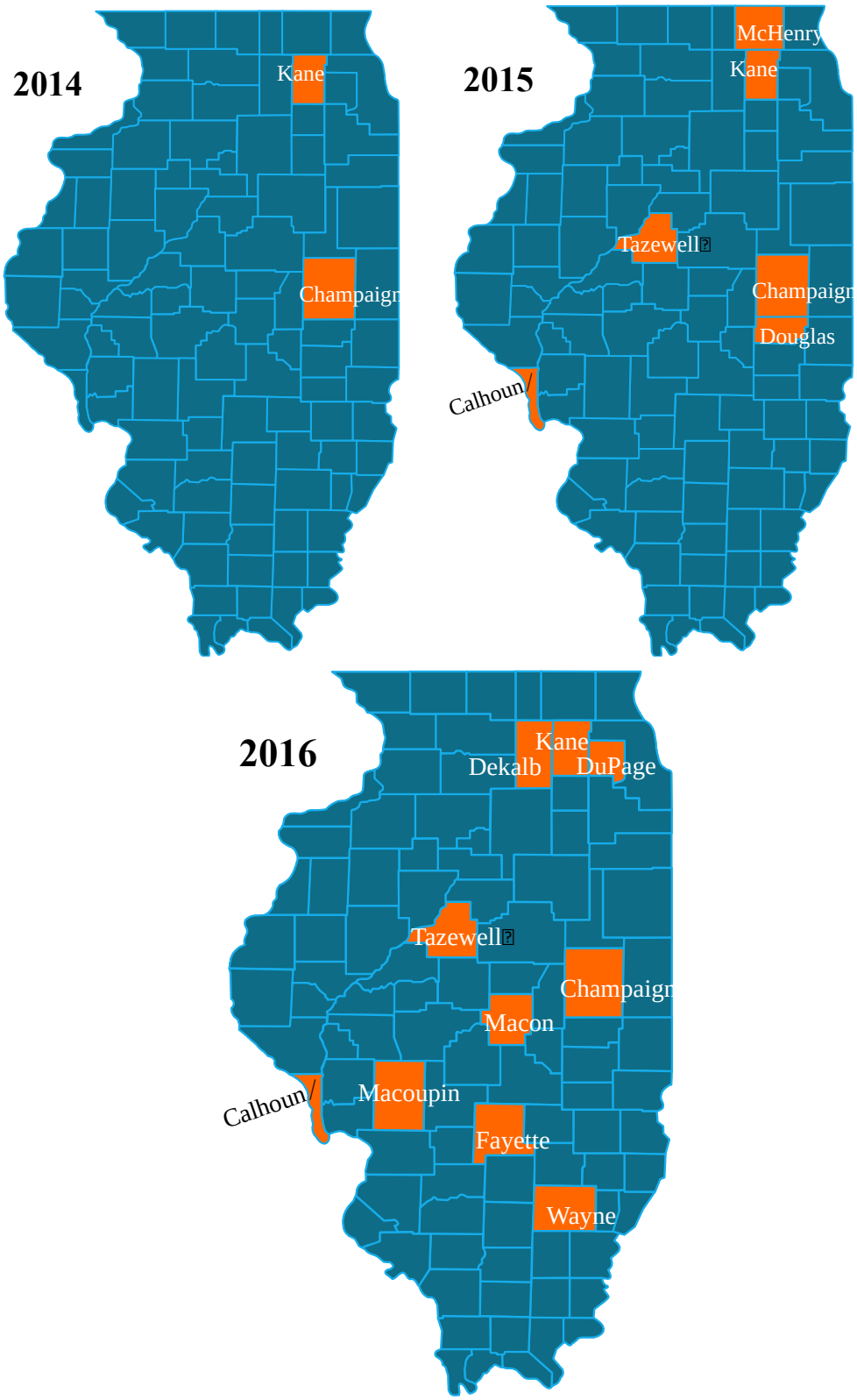

Figure 2.2. Counties (in orange) in Illinois (in orange) where leaf samples of cucurbit crops with powdery mildew were collected in 2014, 2015, and 2016.

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CHAPTER 3
DETERMINE GENETIC AND PATHOGENIC VARIATIONS AMONG ISOLATES OF POWDERY MILDEW FUNGUS OF CUCURBITS

ABSTRACT

This study was conducted to determine the genetic and pathogenic variations among isolates of *Podosphaera xanthii* collected from Illinois and some other cucurbit growing areas in the United States (US), Chile, and Italy. Using the reduced-representation library sequencing technique of genotyping by sequencing (GBS), we found 2266 SNPs for 160 *P. xanthii* isolates tested. Both non-hierarchical K-means clustering and hierarchical clustering revealed low genetic diversity of isolates collected from New York and Italy, but the results suggest a high genetic diversity of the isolates collected in the Midwest and west coast states in the US. Significant variation ($P < 0.0001$) was found in the pathogenicity among 37 isolates of *P. xanthii* tested. In addition, the pathogenicity of 28 isolates from Illinois, collected in different years and from different hosts and locations, were significantly ($P < .0001$) different.

INTRODUCTION

Breeding powdery mildew resistant cucurbit cultivars is greatly dependent on the knowledge of pathogenicity of this disease in a certain production region. Pathogenicity refers to a qualitative trait of genetic capability of a microorganism to cause disease (Sacristan and Garcia-Arenal, 2008). There are limited studies regarding the pathogenic variation of powdery mildew fungi on cucurbits. Information on genetic variation helps us to better understand the evolutionary process and genetic linkage, in order to develop effective management strategies of plant diseases (Burdon, 1993). The genetic variations of powdery mildew pathogens from cucurbits in Illinois and their relationship with geographic origins, host, and variations in

pathogenicity have not been investigated. Genotyping-by-sequencing (GBS) has become a popular approach to assess the genetic diversity even in the absence of reference genome. In this method, a GBS Illumina library is prepared by digesting genomic DNA with restriction enzymes, followed by ligating barcode adapters onto the sticky ends of the digested PCR products. Ligation products were cleaned and amplified before sending for Illumina genomic sequencing (Elshire el al. 2011). Clustering analysis is a commonly used method for analyzing the genetic variations (James et al. 2013).

MATERIALS AND METHODS

Isolate collection

The investigated started with genotyping 160 isolates of *Podosphaera xanthii* in this study. The isolates were collected from six cucurbits species: *Cucumis melo* (cantaloupe), *Cucurbita maxima* (winter squash), *Cucurbita moschata* (processing pumpkin), *Cucurbita pepo* (jack-o-lantern pumpkin), *Cucumis sativus* (cucumber), and *Lagenaria siceraria* (gourd) during 2014-2016. The isolates were from California (CA), Illinois (IL), Indiana (IN), Michigan (MI), New York (NY), Pennsylvania (PA), Texas (TX), Washington (WA), and Wisconsin (WI) in the United States (US); Chile; and Italy. DNA of the isolates from NY were provided by Margaret McGrath from Cornell University, and DNA of the isolates from Chile and Italy were provided by Alessandro Pirondi from the Universita degli Studi di Bologna in Italy.

Conidia from single, small, and discrete colonies were considered genetically homogeneous (Nicot et al., 2002). Pure colonies were prepared by using the pipette inoculation method. Cotyledons of cucumber ‘Bush Crop’ (Rupp Seeds Inc., Wauseon, Ohio, US), a cultivar susceptible to powdery mildew, were harvested one week after sowing seeds, and the surface of

the harvested cotyledons were disinfested by soaking in 0.6% sodium hypochlorite for 5 sec followed by washing twice with sterile distilled water (SDW). The surface-disinfested cotyledons were placed on 90 mm-diameter Petri dishes containing 20 ml water agar with antibiotic (WAA) (18 g agar, 20 mg pimaricin in 1,000 ml distilled water) with the adaxial surface facing up. Cotyledons in the disheswere air dried at room temperature (22°C) in a biological safely hood before inoculating with *P. xanthii*.

The pipette-inoculation method was used to inoculate cotyledons. Using a p10 pipette with 1.5 µL SDW, the plunger of the pipette was pressed to the first stopping point to create a water drop that hung the tip. Then, the water droplet was droplet was used to pick up a clump of conidia by touching a single developed single powdery mildew colony. The water droplet with conidia was quickly deposited onto a cotyledon in the Petri dish. By releasing the pipette plunger, the water droplet was quickly drawn back into the pipette tip, leaving only the clump of conidia on the cotyledon. The grown colony on the cotyledon leaf was considered a pure culture of the powdery mildew fungus. The purified cultures of *P. xanthii* isolates on cotyledons were incubated at 25°C with 12 h light/ 12 h dark cycle for two weeks and were subcultured every two weeks to maintain active cultures. Two-week old cultures on cotyledons were always used for DNA extraction and pathogenicity evaluation.

Determining genetic variation using genotyping-by-sequencing (GBS)

Mycelia and conidia of each isolate were scraped from two-week-old cultures grown on cotyledon, and the DNA was extracted using the E.Z.N.A MicroElute Genomic DNA kit (Omega Bio-Tek Inc; Norcross, Georgia, US). DNA of 160 isolates were tested for their nucleic acid concentration using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA,

US), and the samples were uniformly diluted to 25 ng/µl for all of the isolates in 96-well plates. A GBS Illumina library was prepared following the steps of digestion, ligation and amplification before sending for Illumina genomic sequencing.

The extracted genomic DNA of all of the isolates was digested using a combination of the restriction enzymes (RE) *Hind*III-HF and *Hin*PI. The restriction reaction mix was prepared in a 20 µl volume per isolate reaction containing 2.0 µl 10×Cutsmart Buffer, 0.1 µl rare cutter (*Hind*III-HF), 0.2 µl common cutter (*Hin*PI), 10.2 µl molecular molecular-grade water, and 7.5 µl of uniformly diluted genomic DNA in 96-well plates for the digestion polymerase chain reaction (PCR) at 37°C for 2 hours and 80°C for 20 minutes. The resulting product was preserved at 10°C until analysis.

During the ligation step, the barcode adapters were attached to the sticky end of the digested PCR products. For the total volume of 30 µl per isolate reaction, 2.0 µl 10×Cutsmart Buffer, 3 µl 10 mM dATP in final concentration of 1 mM, 0.1 µl concentrated T4 DNA ligase, 0.5 µl of 10 uM Adapter2, 4.4 µl molecular biology-grade water, and 20 µl digestion PCR product were mixed in each of the wells of 96-well plates. The ligation PCR was carried out at 25°C for 2 hours, and 65°C for 20 minutes. The resulting product was preserved at 10°C until the samples were analyzed.

Ligation PCR products of all 160 isolates were pooled and cleaned up using the AMPure XP beads. The cleaned restriction/ligation product was amplified in the total volume of 50 µl containing 3 µl template product, 2 µl of 10 µM Illumina forward and reverse primer mix, 25 µl 2×Phusion Master Mix, and 20 µl water in a PCR test at 98°C for 30 sec, followed by 15 cycles at 98°C for 10 sec, 68°C for 30 sec, and 72°C for 30 sec, ending with 72°C for 5 min. The resulting product was preserved at 4°C until analysis. The amplified PCR product was cleaned

up using AMPure XP beads, determined for average size and concentration using Agilent DNA7500 chip Bioanalyzer (Agilent Technologies, Inc. Santa Clara, CA, US), and diluted to 10 nmol in LIB buffer [LIB Buffer: 10mM Tris-HCL (EB) with 0.05% Tween-20]. The final product was submitted to the University of Illinois W.M. Keck Center for single-end 100 nt Illumina sequencing on a HiSeq 4000 in one lane.

Prepared DNA sequences were aligned and analyzed for single nucleotide polymorphism (SNPs) using the TASSEL3.0 Universal Network Enabled Analysis Kit (UNEAK) pipeline (Elshire et al., 2011). The minimum minor allele frequency (mnMAF) was set at 0.05; the maximum minor allele frequency (mxMAF) at 0.5; the minimum call rate (mcC) at 0; and maximum call rate (mxC) was set at 1. After detecting the putative SNPs in 160 isolates, SNPs found in less than 89 isolates and the isolates with less than 50% of sites present were removed in TASSEL (TASSEL5, Buckler Lab at Cornell University). The final filtered data was exported in a Hapmap format and analyzed in R Studio.

Non-hierarchical K-means clustering

Non-hierarchical K-means clustering with the Euclidean distance method was applied to the isolates × SNPs data matrix. In addition, the degree of genetic diversity among the filtered isolates was estimated by calculating the pairwise-fixation index (Fst). The pairwise Fst computed Nei's pairwise Fst between all pairs of populations in different clusters calculated from the non-hierarchical K-means clustering.

Hierarchical clustering

Hierarchical clustering with the Euclidean distance method was applied to all of the filtered isolates × SNPs data matrix. In addition, hierarchical clustering was also applied to the filtered Illinois isolates × SNPs data matrix. For the Illinois isolates, the collection location latitude ≤ 39.30°N was classified as "Southern", which included Fayette, Macoupin, and Richland Counties; the collection location latitude > 39.30°N and ≤ 41.03°N was classified as "Central", which included Champaign, Douglas, Marshall, Mason, and Tazewell Counties; and the collection location latitude > 41.03°N was classified as "Northern", which included DeKalb, Kane and McHenry Counties. Accordingly, 13, 33, and 18 isolates were identified as Southern, Central, and Northern, respectively. The Illinois isolates were collected from five cucurbit hosts including *Cucumis melo*, *Cucurbita maxima*, *Cucurbita moschata*, *Cucurbita pepo*, and *Cucumis sativus*. The associations of genetic variation with the geographical origins and host origins of collected samples were analyzed using R Studio (Version 1.0.44).

Pathogenic variation

Thirty-seven isolates, including 2, 1, 28, 2, 2, and 2 isolates from CA, IA, IL, IN, MI, and TX, respectively, were included in this study. Illinois isolates were collected from multiple hosts in various locations throughout the state in 2014, 2015, and 2016. The isolates from other states were collected from *Cucurbita maxima*, *Cucurbita pepo*, and *Cucumis sativus* in 2016.

A cotyledon leaf bioassay was conducted to assess the pathogenicity of the 37 isolates. As previously described, cotyledons of cucumber 'Bush Crop' were harvested from greenhouse-grown seedlings, surface disinfested and placed in 90 mm diameter Petri dishes containing WAA with adaxial surface facing up. Cotyledons in the dishes were air dried at room temperature

(22°C) in a biological safely cabinet before inoculating with *P. xanthii*. The pipette inoculation method was used to inoculate cotyledons for the pathogenicity tests. For each isolate, two dishes with a total of six cotyledons were inoculated. The dishes were incubated in a growth chamber at 25˚C with 16 h light/ 8 h dark for 12-14 days. Using the software ImageJ (version 1.49, National Institutes of Health, US), *P. xanthii* infected area (IA) in cm^2 on cotyledon leaves was recorded. The experiment was repeated five times.

IA values developed by testing 37 isolates were compared using analysis of variance (ANOVA). If the IA values were significantly different, the means were separated and compared using Fisher's least significant difference (LSD) at $P = 0.05$. To determine the relationship between IA values and the host species, locations, and year of collection, a linear model was constructed using the IA value as the dependent variable, and host, location (latitude, longitude, and the interaction of latitude and longitude), and year of collection as independent variables. The host and year were set as categorical variables, while the latitude and longitude were set as continuous variables. If the model was significant, the means were also separated and compared using Fisher's LSD at $P = 0.05$. All statistical analyses for this part were carried out in R Studio.

RESULTS

SNP detected

The library produced over 380 million reads with perfect quality scores from start to finish. We detected a total of 77,019 putative SNPs in all genotyped isolates after calling SNPs without a reference genome using UNEAK pipeline. Using TASSEL 5.0, we removed the SNPs found in less than 89 isolates and removed the isolates with data less than 50% of the scored nucleotide sites. Eventually we obtained 160 isolates with 2,266 SNPs.

Non-hierarchical K-means clustering

A non-hierarchical K-means clustering was applied on the 160 isolates × 2266 SNPs matrix. Three clusters were identified with 23, 29, and 108 isolates in clusters 1, 2, and 3, respectively (Table 3.2). Among the 113 isolates collected from *Cucurbita pepo*, 81 (71%) of the isolates were found in cluster 3. Also, 10 of the 14 (71%) isolates collected from *Cucumis sativus* were found in cluster 3. Among the 64 isolates from Illinois, 30 (47%) isolates were found in cluster 3. Similarly, 32 of 34 (94%) and eight of nine (89%) isolates from NY and Italy, respectively, were found in cluster 3. A pairwise Fst of the non-hierarchical K-means clustering showed significant genetic variation among the clusters (Table 3.3).

Hierarchical clustering

A hierarchical clustering analysis on the distance matrix of 160 isolates × 2266 SNPs identified seven clades (Figure 3.1). The identified seven clades were color-coded as black, orange, pink, red, grey, blue, and green. Nine isolates from Italy and one isolate from Chile were grouped in clade black, and all isolates from the US grouped in the other six clusters. Among the isolates from New York, 30 of 34 (88%) isolates were found in clade blue. We did not find any clustering trend for the isolates collected in other states (such as CA and IL) in the US, which suggested a high genetic variation among isolates from the US. We also did not find a clustering trend based on the hosts. Sixty-four isolates from Illinois, with 2266 SNPs, grouped in five clades (Figure 3.2). Although these isolates had high genetic variation, we did not find any clustering trend among host or location of collection.

Pathogenic variation

Since there was no significant difference in variances among the pathogenicity experiments, IA values of six experiments were combined for analysis. There was a significant difference ($P < 0.0001$) among the IA values of the 37 isolates tested for their pathogenicity (Table 3.4). Since the model was significant, the means of each level of year and host were compared and separated by Fisher's LSD method at $P = 0.05$. Mean separation showed that the isolate ILV1_1_16 from Illinois had the highest IA value (1.03 cm^2) and isolate ILV4_2_16 (also from Illinois) had the lowest IA value (0.59 cm^2) (Table 3.5). Overall, there were significant differences among IA values of the 28 tested isolates from Illinois.

For the 28 tested Illinois isolates, we found that the year, host, latitude, longitude, and latitude × longitude of locations of sample collection had significant ($P < 0.0001$) effects on pathogenicity of the isolates (Table 3.6). Since pathogenicity values were significant, the AI values for years and hosts were compared ($P = 0.05$). Mean separation showed that the isolates collected from 2014 had significantly ($P < 0.0001$) higher mean pathogenicity value (IA = 0.87 cm^2) than those of the isolates collected in 2015 (IA = 0.75 cm^2) and 2016 (IA = 0.76 cm^2) (Table 3.7). Mean separation showed that the isolates collected from *Cucumis sativus* had the highest pathogenicity value (IA = 0.87 cm^2) while the isolates collected from *Cucurbita pepo* had the lowest pathogenicity (IA = 0.73 cm^2) and were significantly ($P < 0.0001$) different (Table 3.7). According to the summary of the linear model, the estimated coefficient of the latitude was 9.29 (± 1.83), the estimated coefficient of the absolute value of longitude was 4.13 (± 0.82), which indicated that the pathogenicity of the isolates was affected by latitude and the absolute value of longitude.

DISCUSSION

In my study, I applied two commonly used clustering methods to determine genetic variation among 160 isolates of *P. xanthii* with 2266 SNPs. The non-hierarchical K-means clustering identified three separate clusters and the hierarchical clustering identified seven clades. Both methods suggested low genetic diversity among isolates collected from NY, Chile, and Italy. In non-hierarchical clustering, all eight isolates from Italy and 32 of 34 isolates from NY were found in the same cluster. In hierarchical clustering, all of the Italian isolates were grouped in the clade black, while 30 out of 34 NY isolates were grouped in the clade blue. When we merged the seven clades into three for the hierarchical clustering at a higher level, we found that the Italian and NY isolates grouped in the same clade, which agrees with the results from the non-hierarchical K-means clustering. The isolate from PA also grouped with the same cluster/clade of the isolates from NY in both clustering analyses. The isolates from the Midwestern and west coast states randomly grouped in three clusters identified by the non-hierarchical K-means clustering, and in six clades by the hierarchical clustering. Both clustering methods suggested that the isolates collected from the Midwestern and west coast states in the US were more genetically diverse than the isolates collected from eastern parts of the US and in Italy. Pirondi, et al. (2015) reported high genetic similarity between isolates of *P. xanthii* from Europe and those from NY, US. Our results agree with their report in that the isolates from Italy and NY were found in the same cluster.

This study revealed significant variation in pathogenicity of *P. xanthii*, especially with high numbers of isolates from Illinois. As an obligate parasite, *P. xanthii* needs a host for its survival. To our knowledge, chasmothecia of *P. xanthii* have not been observed in Illinois and other parts of the US. During winter months, cucurbits are not grown in open fields in Illinois

and other Midwestern states. Therefore, the primary inoculum of *P. xanthii* in Illinois is either blown in from southern states in the US to Illinois or comes from indoor cucurbit production. Conidia of *P. xanthii* are airborne and can be carried long distances by wind (Glawe, 2008). Based on our findings, the isolates collected from the Midwestern and west coast states in the US were more genetically diverse than the isolates collected from eastern parts of the US, which suggests *P. xanthii* travels more between Midwestern and west coast states, but travels less between eastern and the other parts of the US. Additional studies are needed to further assess genetic and pathogenic variations among *P. xanthii* isolates in other states in the US. Such a study will provide more evidence about how genetic and pathogenic variation on isolates in the Midwest and western states affected by the location of overwintering and sources of inoculum.

Another possibility of the initial inoculum of *P. xanthii* in Illinois and other Midwestern states is that *P. xanthii* overwinters on other perennial host plants, where it could produce chasmothecia. Some reported host plants of *P. xanthii* (and its synonyms *P. fusca*, *P. phaseoli*, *Sphaerotheca fusca*), are plants in the families *Asteraceae*, *Fabaceae*, *Orobanchaceae*, *Solanaceae* and *Verbenaceae* (http://www.erysiphales.wsu.edu). For example, it has been reported that a weed named *Calendula arvensis* is colonized by *P. xanthii* (Fernández-Ortuño, et al. 2008). Additional research on the of timeline of hosts infected by different inoculum sources might provide information on the primary inoculum of *P. xanthii*.

Since the sexual reproduction of *P. xanthii* were not found, thus no meiosis reproduction and crossing-over of chromosome segments. Genetic diversity in the fungus may result from mutation and/or parasexuality in its hyphae. Powdery mildew of cucurbits is mainly managed by applications of fungicides, and resistance development in the pathogen to several fungicides has

been reported (McGrath, 2001). Further research on the biology of *P. xanthii* on cucurbits could provide information for effective management of the pathogen.

Table 3.1. Hosts, locations and number of collected and genotyped isolates of *Podosphaera xanthii*.

Host species	Number of collected isolates from each location[z]											Total
	CA	IL	IN	MI	NY	PA	TX	WA	WI	Chile	Italy	
Cucumis melo		4									4	8
Cucurbita maxima	3	5	1	3								12
Cucurbita moschata		6		2								8
Cucurbita pepo	10	42	2	5	34	1	4	5	7	1	2	113
Cucumis sativus		7					4				3	14
Lagenaria siceraria								1				1
Unknown	4											4
Total	17	64	3	10	34	1	8	6	7	1	9	160

[z] CA = California; IL = Illinois; MI = Michigan; NY = New York; PA = Pennsylvania; TX = Texas; WA = Washington state; WI = Wisconsin.

Table 3.2. Non-hierarchical K-means clusters identified for tested isolates of *Podosphaera xanthii* with 2266 SNPs.

	Number of isolates in each cluster			Total isolate no.
Hosts	**Cluster 1**	**Cluster 2**	**Cluster 3**	
Cucumis melo	3	1	4	8
Cucumis sativus	1	3	10	14
Cucurbita maxima	2	3	7	12
Cucurbita moschata	2	1	5	8
Cucurbita pepo	14	18	81	113
Lagenaria siceraria	0	1	0	1
Unknown	1	2	1	4
Total	23	29	108	160
Locations				
California	4	6	7	17
Illinois	19	15	30	64
Indiana	0	0	3	3
Michigan	0	0	10	10
New York	0	2	32	34
Pennsylvania	0	0	1	1
Texas	0	1	7	8
Washington[z]	0	1	5	6
Wisconsin	0	2	5	7
Chile	0	1	0	1
Italy	0	1	8	9
Total	23	29	108	160

[z] Washington State.

Table 3.3 Pairwise Fst values of three clusters found in the non-hierarchical K-means clustering for the 160 isolates of *Podosphaera xanthii* with 2266 SNPs.

	Cluster 1	**Cluster 2**	**Cluster 3**
Cluster 1	0.0000	0.4097[z]	0.3185
Cluster 2		0.0000	0.3149
Cluster 3			0.0000

[z] > 0.2 pairwise Fst values indicate significant genetic variations among the clusters.

Table 3.4. Infected area (IA) of cotyledon leaves following inoculation with *Podosphaera xanthii* isolates.

Isolate	Year	Host	State	County	Latitude	Longitude	IA(cm^2)
ILV1_1_16	2016	*Cucurbita moschata*	IL	Fayette	38.96	-89.09	1.03 a[z]
ILN3_2_16	2016	*Cucurbita pepo*	IN	DuPage	39.82	-85.85	0.98 ab
TX3_2	2016	*Cucumis sativus*	TX	Potter	35.22	-101.83	0.98 ab
IL17_JPGH	2014	*Cucumis sativus*	IL	Champaign	40.12	-88.24	0.97 a-c
IA1-2	2016	*Cucurbita pepo*	IA	Muscatine	41.42	-91.04	0.94 a-c
IL06_QGH	2014	*Cucumis sativus*	IL	Champaign	40.12	-88.24	0.90 a-d
TX1_2	2016	*Cucurbita pepo*	TX	Potter	35.22	-101.83	0.88 b-d
IL14_JP081603_3	2015	*Cucurbita pepo*	IL	Calhoun	39.17	-90.66	0.86 c-f
IN3_1	2016	*Cucurbita pepo*	IN	Hancock	39.82	-85.85	0.86 c-f
IL04_CL0829_3	2015	*Cucumis melo*	IL	Champaign	40.12	-88.24	0.84 c-g
IL22	2015	*Cucurbita moschata*	IL	Champaign	40.12	-88.24	0.84 c-g
ILD1_16	2016	*Cucumis melo*	IL	Macon	39.84	-88.95	0.84 c-g
MI3_1	2016	*Cucurbita pepo*	MI	Berrien	42.12	-86.45	0.84 c-g
IL10_JP0816_4	2015	*Cucurbita pepo*	IL	Calhoun	39.17	-90.66	0.83 c-g
IL18_NP0906_2	2015	*Cucurbita pepo*	IL	Champaign	40.12	-88.24	0.80 d-h
IL11_JP081601_2	2015	*Cucurbita pepo*	IL	Calhoun	39.17	-90.66	0.79 d-h
IL09_JP0809_3	2015	*Cucurbita pepo*	IL	McHenry	42.17	-88.43	0.76 d-h
CA8-1	2016	*Unknown*	CA	Monterey	36.68	-121.66	0.75 e-h
IL05_Q5	2014	*Cucumis sativus*	IL	Kane	40.12	-88.24	0.75 e-h
ILN2	2016	*Cucurbita pepo*	IL	DuPage	41.75	-88.15	0.75 e-h
IN3_2	2016	*Cucurbita pepo*	IN	Hancock	39.93	-85.85	0.74 f-h
IL08_JP0809_2	2015	*Cucurbita pepo*	IL	McHenry	42.17	-88.43	0.73 f-h
IL20_PP082401_2	2015	*Cucurbita moschata*	IL	Tazewell	40.57	-89.64	0.73 f-h
IL19_NP0906_3	2015	*Cucurbita pepo*	IL	Champaign	40.12	-88.24	0.72 g-i
IL23	2015	*Cucurbita moschata*	IL	Champaign	40.12	-88.24	0.72 g-i
MI6_1	2016	*Cucurbita pepo*	MI	Berrien	42.12	-86.45	0.72 g-i
IL21_PP082404_1	2015	*Cucurbita moschata*	IL	Tazewell	40.41	-89.64	0.71 g-i
ILD2_16	2016	*Cucumis melo*	IL	Macon	39.84	-88.95	0.70 h-i

Table 3.4 (cont.)

IL28_YS0816_3	2015	*Cucurbita pepo*	IL	Douglas	39.76	-88.21	0.69 h-j
IL30_YS0829_1	2015	*Cucurbita pepo*	IL	Champaign	40.12	-88.24	0.69 h-j
CA9-1	2016	*Cucurbita maxima*	CA	San Benito	36.85	-121.40	0.68 h-j
IL24_P08230202	2015	*Cucurbita pepo*	IL	Kane	42.10	-88.53	0.68 h-j
IL29_YS082303_1	2015	*Cucurbita pepo*	IL	McHenry	42.17	-88.43	0.68 h-j
IL33_YS0725_2	2015	*Cucurbita pepo*	IL	Douglas	39.68	-88.31	0.67 h-j
IL5_16	2016	*Cucurbita pepo*	IL	Wayne	41.95	-88.24	0.60 ij
IL7_16	2016	*Cucurbita pepo*	IL	Tazewell	40.57	-89.64	0.59 j
ILV4_2_16	2016	*Cucurbita pepo*	IL	Fayette	38.96	-89.09	0.59 j

[z] Each value represents the average of measurements of eight cotyledon leaves. Values followed with the same letter are not significantly different from each other according to the Fisher's Least Significant Difference test at $P = 0.05$.

Table 3.5. Analysis of variance for the infected area (IA) values (cm^2) for 37 *Podosphaera xanthii* isolates.

Term	**DF[z]**	**Sum square**	**Mean square**	**F value**	***P* value**
Isolate	36	19.21	0.53	6.64	<0.0001*
Residual	1363	109.47	0.08		

[z] DF = degree of freedom.

Table 3.6. Analysis of variance for the infected area (IA) values (cm^2) for the 28 *Podosphaera xanthii* isolates collected from Illinois.

Term	**DF[z]**	**Sum square**	**Mean square**	**F value**	***P* value**
Year[y]	2	1.10	0.55	6.51	0.0016*
Host[x]	2	1.68	0.84	9.95	<.0001*
Latitude[wv]	1	1.03	1.03	12.15	0.0005*
Longitude[v]	1	1.16	1.16	13.71	0.0002*
Latitude: Longitude	1	2.19	2.19	25.89	<.0001*
Residuals	960	81.23	0.08		

[z] Degree of freedom.
[y] Isolates were collected in 2014, 2015, and 2016.
[x] Isolates were collected from four cucurbit hosts including *Cucumis melo*, *Cucurbita moschata*, *Cucurbita pepo*, and *Cucumis sativus*.
[w] Absolute value of longitude was used in this model.
[v] Latitude and longitude information were collected from the "Get Latitude and Longitude" website (http://www.latlong.net).

Table 3.7. Comparison of mean infected area (IA) values (cm^2) of isolates collected from Illinois.

Year	**Number of isolates**	**IA[z]**
2014	3	0.87 a
2016	8	0.76 b
2015	17	0.75 b
Host		
Cucumis sativus	3	0.87 a
Cucurbita moschata	5	0.84 ab
Cucumis melo	3	0.79 b
Cucurbita pepo	17	0.73 c

[z] Means followed by the same letter are not significantly different according to the Fisher's Least Significant Difference ($P = 0.05$).

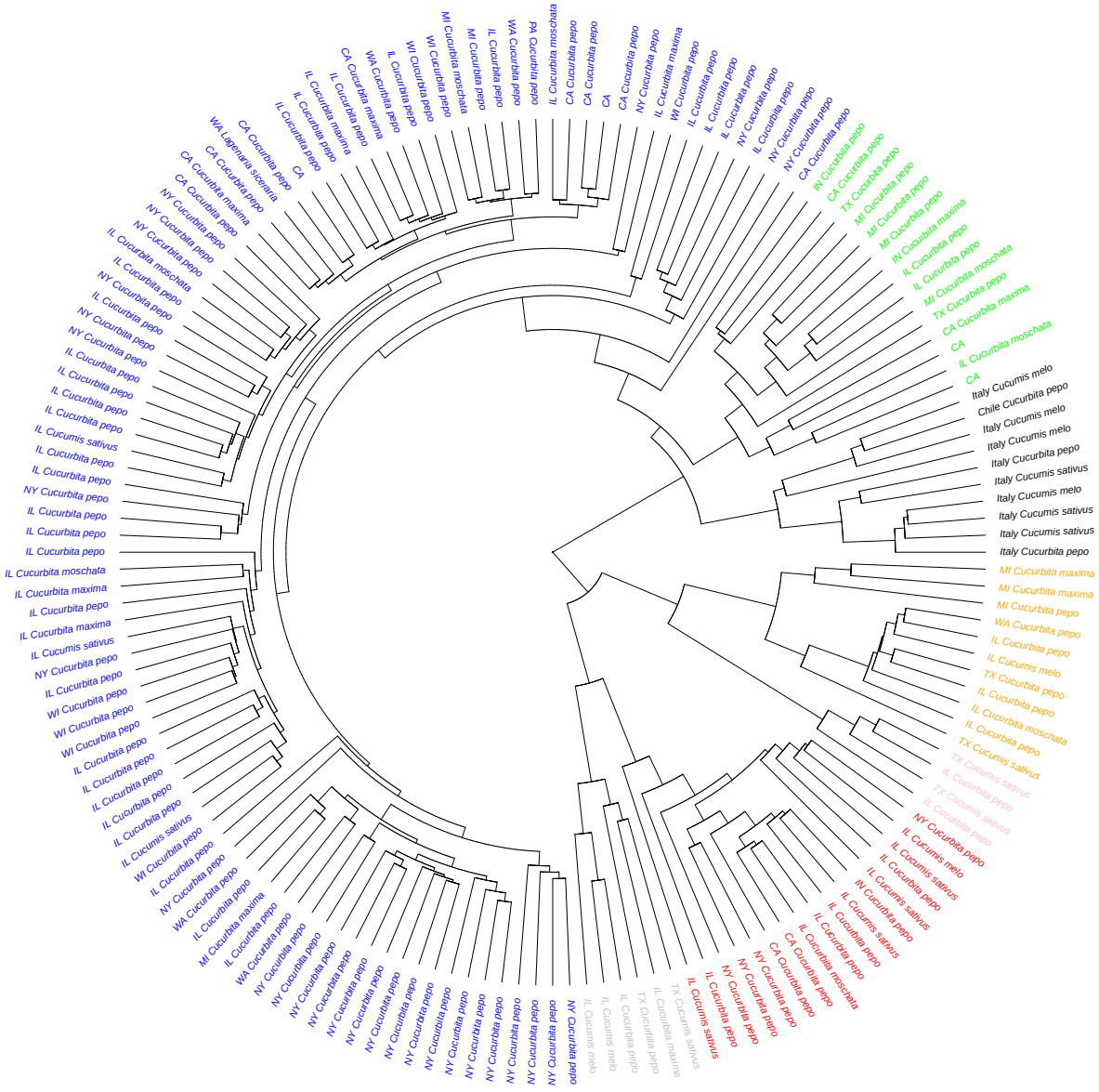

Figure 3.1. Hierarchical clustering of the distance matrix of 160 isolates of *Podosphaera xanthii* with 2,266 SNPs, which clustered in seven distinct clades. The phylogenetic tree is labeled with the host names and locations of collected isolates, color-coded as black, orange, pink, red, grey, blue, and green for each of seven different clades. One isolate from Chile and nine isolates from Italy were clustered in the black clade, while all of the isolates from the United States clustered in the other six clades.

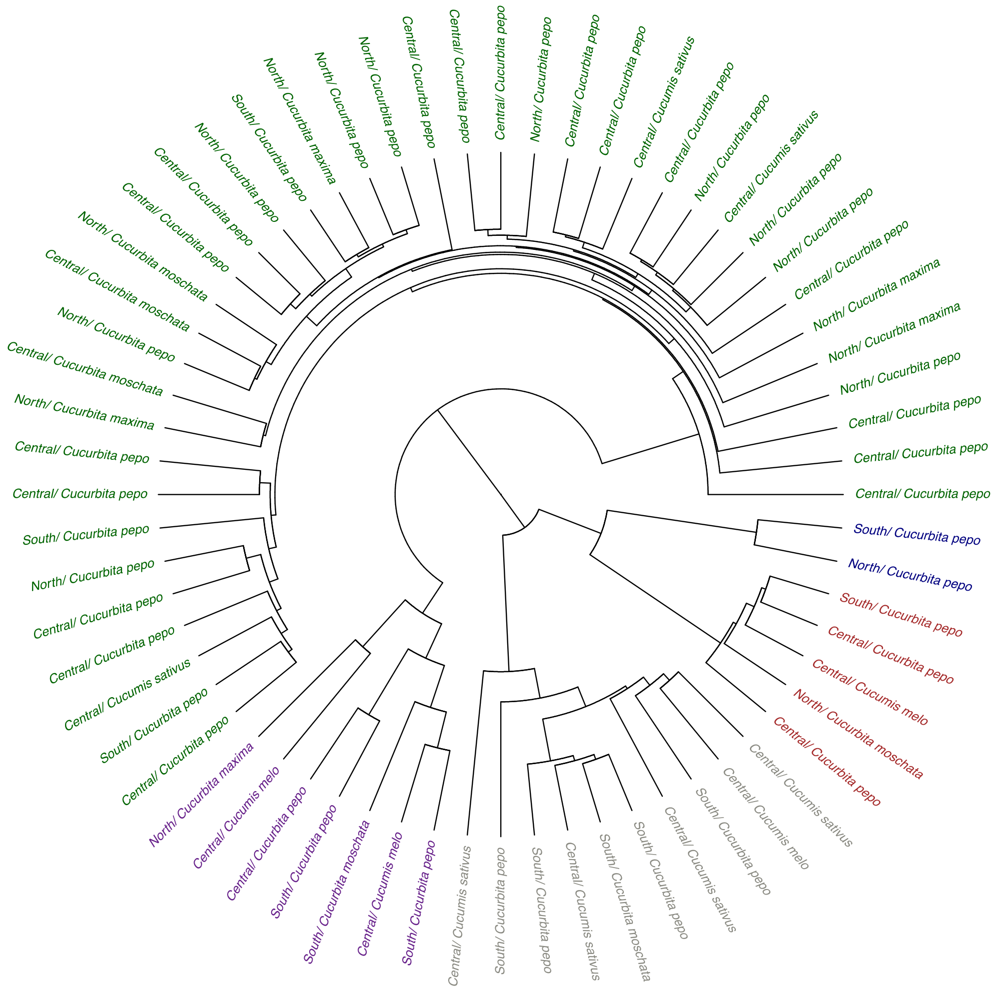

Figure 3.2. Hierarchical clustering of the distance matrix of 64 isolates of *Podosphaera xanthii* from Illinois with 2,266 SNPs, which were clustered in five distinct clades, color-coded as dark green, purple, ivory, brown and navy. This phylogenetic tree is labeled by regions (south, central, and north) of Illinois and five hosts (*Cucumis melo*, *Cucurbita maxima*, *Cucurbita maxima*, *Cucurbita pepo*, and *Cucumis sativus*).

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CHAPTER 4
DETERMINE THE SENSITIVITIES OF CUCURBIT POWDERY MILDEW FUNGUS TO MAJOR POWDERY MILDEW FUNGICIDES

ABSTRACT

Powdery mildew causes up to 50% yield losses in cucurbits in Illinois. Fungicide application is the major method for managing this disease. Since 2010, growers in Illinois have complained about failures of some fungicides to effectively control powdery mildew in cucurbit fields. This research was conducted to evaluate the sensitivity of cucurbit powdery mildew pathogen in Illinois and other states in the United States (US) to fungicide groups commonly used to control powdery mildew on cucurbits, including demethylation inhibitors (DMI), quinone outside inhibitor (QoI), succinate dehydrogenase inhibiting (SDHI), quinolines, and cyflufenamid (a new fungicide with unknown mode of action). In 2015 and 2016, 28 *Podosphaera xanthii* isolates, were collected from cucurbit fields in 10 counties in Illinois, and nine isolates were collected from cucurbit fields in five other states in the US. These isolates were then evaluated for their sensitivty to the DMI fungicude triflumizole (Procure 480SC), the QoI fungicie azoxystrobin (Quadris 2.08SC), the SDHI fungicide penthiopyrad (Fontelis 1.67SC) , and cyflufenamid (Torino 0.85SC) in laboratory based assays. Also, these fungicides and the quinolone fungicide quinoxyfen (Quintec 250SC) were evaluated for their efficacy in controlling of powdery mildew of jack-o-lantern pumpkin 'Howden' in the field during the 2011-2017 growing seasons. Our studies in the laboratory showed that the isolates were highly sensitive to triflumizole and cyflufenamid, but showed reduced sensitivities to azoxystrobin and penthiopyrad. Reduced sensitivity to azoxystrobin was also found in the field evaluations. Field trials also showed significant differences ($P<0.0001$) in efficacy among various rates of azoxystrobin (Quadris 2.08SC and Torino 0.85SC) in controlling powdery mildew of pumpkin.

INTRODUCTION

Resistant cucurbit cultivars to powdery mildew have been constantly overcome by more virulent pathogen strains (McGrath, 2001). Resistance development in *P. xanthii* to fungicides is a serious problem due to the intensive application of fungicides as a major management strategy for cucurbit powdery mildew control (Bellón-Gómez et al., 2014; McGrath, 2001). Several fungicides with different modes of actions have been used to manage powdery mildew on cucurbits in Illinois and other Midwestern states, which include demethylase inhibitors (DMI), Quinone outside Inhibitors (QoI), succinate dehydrogenase inhibitor (SDHI), quinolones and cyflufenamid with unknown mode of action. While remaining their effectiveness for the majority of the times, resistance has been reported in DMI fungicides (Adams et al., 2007; Lóopez-Ruiz et al., 2010; McGrath, 2001), SDHI (Miyamoto et al., 2010), Quinoxyfen (Hollomon et al., 1997) and cyflufenamid (Pirondi et al., 2015). Resistance of powdery mildew fungi to QoI fungicides has developed quickly due to the intensive use of these fungicides and its long use on the market (Ishii et al., 2001; Fernandez-Ortuno et al., 2006; Fernández-Ortuño et al., 2008; McGrath and Shishkoff, 2003). The DMI fungicide triflumizole formulated as Procure 480SC, QoI fungicide azoxystrobin formulated as Quadris 2.08SC, SDHI fungicide penthiopyrad formulated as Fontelis 1.67SC, cyflufenamid formulated as Torino 0.85SC and quinexyfen formulated as Quintec 250SC have all been extensively used for managing powdery mildew of cucurbits. The understanding of the effectiveness of these fungicides in Illinois plays a major role in developing strategies in cucurbit powdery mildew management.

MATERIALS AND METHODS

Isolate collection and maintenance

Thirty-seven leaf samples with powdery mildew were used in this study. The samples were collected from cucumbers (*Cucumis sativus*), cantaloupe (*Cucumis melo*), jack-o-lantern pumpkins (*Cucurbita pepo*), processing pumpkins (*Cucurbita moschata*), and winter squash (*Cucurbita maxima*). All of the samples were collected from commercial cucurbit fields in California (CA), Indiana (IN), Illinois (IL), Iowa (IA), Michigan (MI), and Texas (TX) in the United States (US) (Figures 4.1 and 4.2) from July through September in 2015 and 2016. Pure colonies of *Podosphaera xanthii* isolates were prepared from each leaf sample on cotyledons of cucumber 'Bush Crop' (Rupp Seeds Inc. Wauseon, Ohio, US). The colonies of this biotrophic fungus were maintained on cucumber cotyledons placed on water agar with antibiotic (WAA) (agar: 18 g; pimaricin: 20 mg; and distilled water： 1,000 ml) in 60 mm diameter Petri dishes. The colonies were subcultured every two weeks.

Fungicide efficacy tests in the laboratory

Commercial formulations of fungicides from five mode of action (FRAC) groups were used in the laboratory studies. Fungicides used included: triflumizole (Procure 480SC, Chemtura, Middlebury, Connecticut), from the demethylation inhibitors (DMI) group; azoxystrobin (Quadris 2.08SC, Syngenta, Greensboro, North Carolina), from the quinone outside inhibitor (QoI) group; penthiopyrad (Fontelis 1.67SC, DuPont, Wilmington, Delaware), from the succinate dehydrogenase inhibiting (SDHI) group; and cyflufenamid (Torino 0.85SC, Gowan, Yuma, Arizona), a newly registered fungicide with an unknown mode of action (U6). Since quinoxyfen caused phytotoxicity (yellowing and necrosis on cotyledons), evaluation of the

efficacy of this fungicide for control of powdery mildew on cucumber in the laboratory was not possible. Therefore, evaluation of effectiveness of quinoxyfen for control of powdery mildew was conducted in the field only.

For Procure 480SC, the concentrations of 0.08, 0.81 and 4.04 µg/ml were tested for isolates collected in 2015 and 2016; for Quadris 2.08SC, the concentrations of 0.46, 4.57, 45.67 and 456.61 µg/ml were tested for isolates collected in 2015 and concentrations of 45.66, 114.15, and 342.46 µg/ml for isolates collected in 2016; for Fontelis 1.67SC, the concentrations of 0.41, 4.08, and 20.41 µg/ml were tested for isolates collected in both 2015 and 2016; and for Torino 0.85SC, the concentrations of 0.0010, 0.0100 and 0.0498 µg/ml were tested for isolates collected in 2015 and 2016.

A modified cotyledon bioassay reported by previous investigators (Bellón-Gómez et al., 2014; Lebeda et al., 2010) was used in this study. Cucumber cultivar 'Bush Crop' was grown in the greenhouse, five days later cotyledons were harvested and surface-disinfested using 0.6% sodium hypochlorite, and washed with sterile distilled water (SDW), as described in chapter 2. Surface-disinfested cucumber cotyledons were treated with the fungicides by soaking them in the prepared concentrations of each fungicide for 2 sec. The fungicide treated cotyledons were transferred onto the WAA in 90 mm diameter Petri dishes with adaxial the surface facing up, and air dried at room temperature in a biological safely cabinet for 1 h before inoculating with *Podosphaera xanthii*. Three plates, each with two cotyledons, were used for each isolate with each concentration of each fungicide. For each treatment, control cotyledons with no fungicide treatment but inoculated with the isolate were used. The experiment was repeated once.

Inoculation of cotyledons was carried out with the pipette inoculation method described in chapter 2. Inoculated cotyledons in the WAA plates were incubated in a growth chamber at

25˚C with 12h light/12 h dark for 12-14 days before preparing images of the cotyledons for determining infection severity.

Using software ImageJ (ImageJ 1.49v, National Institutes of Health, Bethesda, Maryland, US), infection area (IA) in cm^2 was recorded from the images. The less sensitive isolates produced larger IA than the more sensitive isolates. The IA values were transformed by adding 10^{-5} and then taking the square root to prepare for statistical analysis. Data from two repeated trials in each year, and data from two years were combined for analysis if the error variances were homogenous after an F Test. Analysis of covariance (ANCOVA) models were fit to test if there were any significant variations in sensitivity among the tested isolates with each fungicide. Values of IA were modeled as the dependent variable with isolates as the categorical factor and concentration as the covariate factor. Least squares mean (LSM) were calculated on the IA values of each isolate from three concentrations of that particular fungicide if there were interaction between the concentration and the isolates. LSM were separated at the highest test concentration for each fungicide using Tukey's pairwise comparison at $P = 0.05$ when ANCOVA indicated that there were significant differences among them. Minimum inhibitory concentrations (MICs) were deduced directly from data. When variations of the sensitivities to the fungicides were detected, the isolates were divided into two groups, the more sensitive group and the less sensitive group, according to the MIC values.

Fungicide efficiency tests in the field

Efficacy of all of five fungicides (Table 4.1) for control of powdery mildew on jack-o-lantern pumpkin 'Howden' was evaluated during the 2011 through 2017 growing seasons. The experiments were conducted at the University of Illinois Vegetable Research Farm in

Champaign, IL. Fungicides were applied with a motorized backpack sprayer (Solo 451 Motorized Mist Blower Backpack, Sindelfingen, Germany) at 5.63 kg/cm^2 using 469 L of water per ha.

The fungicides tested in the field were: 1) triflumizole (Procure 480SC, at 0.583 L per ha) in 2013, 2014, 2015 and 2016; 2) azoxystrobin (Quadris 2.08SC, at 1.1 L per ha) in 2011, 2012, and 2015; 3) penthiopyrad (Fontelis 1.67SC, at 1.17 L per ha) in 2011, 2012, 2015, and 2016; 4) quinoxyfen (Quintec 2.08SC, at 0.437 L per ha) in 2013, 2014, 2015, and 2016; and 5) cyflufenamid (Torino 0.85SC, at 0.248 L per ha) in 2015 and 2016.

In 2017, a comprehensive trial was carried out to not only evaluate the effectiveness of each of the five fungicides alone, but also assess the differences of each of the fungicides at three different rates for control of powdery mildew on pumpkin 'Howden'. The 2017 field trial included the following treatment: 1) triflumizole (Procure 480SC), at 0.292, 0.438, and 0.584 L per ha; 2) azoxystrobin (Quadris 2.08SC), at 0.802, 0.966, and 1.130 L per ha; 3) penthiopyrad (Fontelis 1.67SC), at 0.875, 1.021, and 1.166 L per ha; 4) quinoxyfen (Quintec 2.08SC), at 0.292, 0.364, and 0.437 L per ha; and 5) cyflufenamid (Torino 0.85SC), at 0.175, 0.211, and 0.248 L per ha. Three rates of each fungicide were based on the lowest, medium, and highest rates recommended on the product labels for control of powdery mildew of cucurbits. Applications of the fungicides began five weeks after seeding and ended two weeks before harvesting the fruit. Eight spray applications of each of fungicides, with each rate, were carried out at weekly intervals.

The soil of the Vegetable Research Farm is a silt clay loam with pH 6.5. Seeds of Jack-o-lantern pumpkin 'Howden' were sown 46 cm apart in single-row plots, each 6 m long (Table 4.9). In 2015, because of heavy rainfalls and loss of some of the originally seeded plots, I had to

re-establish all of the plots using 18-day-old transplants grown in the greenhouse. The plots were spaced 9 meters apart in a randomized complete block design with four replications for each treatment. During the season, weeds were controlled by cultivation and hand weeding. Applications of fungicides began 5-6 weeks after sowing seeds and continued at 7-day intervals until 2-3 weeks prior to harvest. The severity of powdery mildew on leaves was rated using the average rating of four randomly chosen leaves, and in each leaf the rating was generated from the visually assessed percentage of total infection area (from 0 to 100%). The assessment was carried out at biweekly intervals from 5-6 weeks after sowing seeds until 1-2 weeks before harvest. Severity of the disease was assessed at four locations of each plot and continued at the same locations throughout the season. At each location, leaves in approximately 1 m^2 were checked for severity of powdery mildew. The area under the disease progress stairs (AUDPS) was calculated using disease severity ratings recorded over time for each block in each fungicide treatment and control (Simko and Piepho, 2012). The area under the disease progress curve (AUDPC) has been a widely used method to assess the disease progress. It breaks up a disease progress curve into a series of trapezoids, and adds up the area of each trapezoids (Jeger and Viljanen-Rollinson, 2001):

$$AUDPC = \sum_{i=1}^{n-1} \frac{y_i + y_{i+1}}{2} \times (t_{i+1} - t_i)$$

Compared to the (AUDPC), the mathematical formula for AUDPS is

$$AUDPS = AUDPC + (\frac{y_1 + y_n}{2} \times \frac{D}{n-1})$$

where y_1 and y_n are the first and last disease ratings, respectively; t_1 and t_n are the times of the first and last ratings, respectively, where $D = t_n - t_1$. After adding 10 to these AUDPS values equal to zero, the values of AUDPS were transformed using logarithm for correction and

analyzed using R studio. For each fungicide, the linear regression model was built using the transformed AUDPS as the dependent variable, and the tested year and fungicide treatment as the independent categorical variables. The efficiency of each fungicide over the years was compared by comparing the transformed AUDPS values from all of the tested years for each fungicide. If the model was significant, the means were separated and compared by Fisher's Least Significant Difference (LSD) at $P = 0.05$.

For each rate of each fungicide evaluated in 2017, the linear regression model was built using the transformed AUDPS as the dependent variable, and the tested rate as the independent continuous variables. The efficiencies of different rates of each fungicide were compared by comparing the transformed AUDPS values from all of the tested rates for each fungicide. If the model was significant, the means were separated and compared by Fisher's LSD at $P = 0.05$.

RESULTS

Fungicide efficiency tests in the laboratory

DMI

Since the error variances from 2015 and 2016 were homogenous, the data from both years were combined for analysis. Significant effects of fungicide concentration, isolate, and their interactions were found by ANCOVA, indicating that there were significant variations ($P < 0.0001$) in sensitivity among the 37 isolates to triflumizole (Procure 480SC) (Table 4.3).

The LSM of IA values of all 37 isolates for all three concentrations (0.08, 0.81, and 4.04 µg/ml) are presented in Table 4.4. At the concentration of 4.04 µg/ml, LSM of IA values of the isolates were compared and ranked from the least sensitive isolate to the most sensitive isolate. Among the 37 isolates tested at the concentration of 4.04 µg/ml, the least sensitive isolate (IA1-

2) produced the highest LSM value of IA (0.77 ± 0.08 cm^2), compared to the most sensitive isolate (ILV19) that produced the lowest LSM value of IA (-0.12 ± 0.08 cm^2). According to the determined MICs of the 37 isolates to triflumizole, two groups of isolates with different sensitivities (less sensitive and more sensitive) can be identified. The less sensitive group was composed of 27 isolates with MIC values equal or larger than 4.04 μg/ml, while the more sensitive group consisted of 10 isolates with MIC values ranging from 0.08 to 0.81 μg/ml (Table 4.2). At the highest tested concentration of 4.04 μg/ml, it was found that, overall, LSM of IA values of the isolates from 2016 ranked higher than those from 2015 (Table 4.4). In 2016, 15 of 17 isolates (88%) were found in the less sensitive group according to the MICs, while only 12 of 20 isolates (60%) were found in the less sensitive group in 2015 (Table 4.2). Both ANCOVA models and MICs values indicated that the isolates from 2016 showed a greater incidence of reduced sensitivity to triflumizole than those from 2015. Overall, we found 27 of 37 isolates (73%) were classified in the less sensitive group. Similarly, 18 of 28 isolates (64%) from Illinois were classified in the less sensitive group (Table 4.8).

QoI

Since the error variances from 2015 and 2016 were homogenous, the data from both of these years were combined for analysis. Significant effects of the concentration, isolate, and their interactions were found by ANCOVA, indicating that there were significant (P <0 .0001) sensitivity variations among the 37 isolates to azoxystrobin (Quadris 2.08SC) (Table 4.3). LSM of IA values of all of the isolates in all three concentrations (0.46, 45.66, and 342.46 μg/ml) are presented in Table 4.5. At the concentration of 342.46 μg/ml, LSM of IA values of 37 isolates were compared and ranked from the least sensitive to the most sensitive (Table 4.5). Among the

37 isolates tested at the concentration of 342.46 μg/ml, the least sensitive isolate (TX3-2) produced the highest LSM value of IA of (0.76 ± 0.11 cm^2), compared to the most sensitive isolate (ILV1-1) that produced the lowest LSM value of IA of (- 0.16 ± 0.1 cm^2).

According to the determined MICs of the 37 isolates to azoxystrobin (Quadris 2.08SC), two groups of isolates with different sensitivities were identified. The less sensitive group was composed of 29 isolates that showed MIC values larger than 342.46 μg/ml, while the more sensitive group consisted of eight isolates that showed MIC values ranging from 0.46 to 45.66 μg/ml (Table 4.2). In 2016, 14 of 17 isolates (82%) were in the less sensitive group compared to 15 of 20 isolates (75%) in the less sensitive group in 2015 (Table 4.2). Both ANCOVA models and MICs values indicated that the isolates from both years showed reduced sensitivities to azoxystrobin (Quadris 2.08SC). Overall, 29 of 37 isolates (78%) were classified in the less sensitive group. Similarly, 20 of 28 isolates (71%) from Illinois were in the less sensitive group (Table 4.8).

SDHI

Since the error variances for the data from 2015 and 2016 were homogenous, data from both years were combined for analysis. Significant effects of the concentration, isolate, and their interactions were found by ANCOVA, indicating that there were significant variations ($P <$ 0.0001) among the sensitivities of the 37 isolates to penthiopyrad (Fontelis 1.67SC) (Table 4.3). LSM of IA values of all of the isolates at three concentrations (0.41, 4.08, and 20.41 μg/ml) are presented in Table 4.6. At the concentration of 20.41 μg/ml, LSM of IA values of 37 isolates ranked from the least sensitive isolate to the most sensitive isolate (Table 4.6). Among the 37 isolates tested at the concentration of 20.41 μg/ml, the least sensitive isolate (ILN3-2) produced

the highest LSM value of IA (0.93 ± 0.07 cm^2), compared to the most sensitive isolate (IL28) that produced the lowest LSM value of IA (- 0.14 ± 0.1 cm^2). Both isolates ILN3-2 and IL28 were from Illinois.

According to the determined MICs of the 37 isolates to penthiopyrad (Fontelis 1.67SC), two groups of isolates (less sensitive and more sensitive) were identified. The less sensitive group was composed of 28 (76%) isolates that showed MIC values equal or larger than 20.41 μg/ml, while the more sensitive group consisted of nine (24%) isolates that showed MIC values of 4.08 μg/ml (Table 4.2). At the highest tested concentration of 20.41 μg/ml, it was found that all of the LSM of IA values of the isolates from 2016 ranked higher than those from the isolates collected in 2015 (Table 4.6). In 2016, all of the 17 isolates (100%) were found in the less sensitive group according to the MICs, while only 11 of 20 isolates (55%) were found in the less sensitive group in 2015 (Table 4.2). Both ANCOVA models and MICs values indicated that the isolates from 2016 showed less senitivity to penthiopyrad (Fontelis 1.67SC) than did the isolates collected in 2015. Overall, we found 28 of 37 isolates (76%) were classified in the less sensitive group. Among 28 isolates from Illinois, 19 isolates (68%) were in the less sensitive group (Table 4.8).

Cyflufenamid (U6)

Since the error variances from 2015 and 2016 were not homogenous, the data from each year were analyzed separately for cyflufenamid (Torino 0.85SC). Significant effects of concentration, isolate, and their interactions were found by ANCOVA, indicating that there were significant variations ($P < 0.0001$) in sensitivities of the isolates to cyflufenamid (Torino 0.85SC), both in 2015 and 2016 (Table 4.3). LSM of IA values of all of the isolates at the three

concentrations (0.0010, 0.0100, and 0.0498 µg/ml) are presented in Table 4.6. At the concentration of 0.0498 µg/ml, LSM of IA values of 20 isolates from 2015 and 17 isolates from 2016 were compared and ranked from the least sensitive isolate to the most sensitive isolate (Table 4.7). In 2015, the isolates produced the LSM of IA ranging from -1.14 to 0.32 cm^2, which were not significantly different from each other. Among the 17 isolates from 2016 tested at the concentration of 0.0498 µg/ml the least sensitive isolate (MI3-1) produced the highest LSM value of IA (0.76 ± 0.07 cm^2) compared to the most sensitive isolate (MI6-1) that produced the lowest LSM value of IA (- 0.01 ± 0.07 cm^2). For all of the 37 isolates, MIC values were identified equal to or larger than 0.0498 µg/ml (Table 4.2).

The LSM of IA values of 20 isolates collected in 2015 showed that all of the isolates were highly sensitive to this fungicide (Table 4.7). At the highest tested concentration (0.0498 µg/ml), 12 of 17 (71%) isolates from 2016 were found with MICs larger than 0.0498, while only four of 20 isolates (20%) were found with MICs larger than 0.0498 in 2015 (Table 4.2). Both ANCOVA models and MICs values indicated that all of the isolates from 2015 were sensitive to cyflufenamid (Torino 0.85SC), while more isolates from 2016 showed reduced sensitivities to this fungicide.

Sensitivity of isolates from Illinois in the laboratory

Sensitivity of *P. xanthii* isolates from northern, central, and southern Illinois were compared. In northern Illinois, of eight isolates tested, seven (88%), eight (100%), and five (63%) isolates were less sensitive to triflumizole (Procure 480SC, DMI group); azoxystrobin (Quadris 2.08SC, QoI group); and penthiopyrad (Fontelis 1.67SC, SDHI group) fungicides, respectively. In central Illinois, of fifteen isolates tested, eight (53%), ten (63%), and nine (60%)

isolates were less sensitive to triflumizole (DMI group), azoxystrobin (QoI group), and penthiopyrad (SDHI group) fungicides, respectively. In southern Illinois, of five isolates studied, three (60%), two (40%), and five (100%) were less sensitive to triflumizole (DMI group), azoxystrobin (QoI group), and penthiopyrad (SDHI group) fungicides, respectively. Among nine isolates tested from other states, all nine (100%) were less sensitive to triflumizole (DMI group), azoxystrobin (QoI group), and penthiopyrad (SDHI group) fungicides.

Fungicide efficiency in the field

Powdery mildew was first observed in the untreated plots in early August every year, and disease severity increased as the season progressed. Severity of powdery mildew was significantly higher ($P < 0.0001$) in unsprayed control plots compared to the treated plots in all seven years (Table 4.10). All of the treatments controlled powdery mildew satisfactorily throughout the season (Table 4.11). Since no significant block effect was found with any of the trials, the block was used as a random replication factor in the data analysis. For all of the fungicides in all years, each of the treatments was compared with the untreated control of that particular year.

There was a significant year and treatment interaction ($P < 0.0001$) (Table 4.10). Fungicide treatment and year combinations were compared and separated for all of the treatments in order to observe if there was any trend of reduced effectiveness over the years (Table 4.11). We found that triflumizole (Procure 480SC), penthiopyrad (Fontelis 1.67SC), quinoxyfen (Quintec 2.08SC) and cyflufenamid (Torino 0.85SC) were effective for control of powdery mildew of pumpkin over the years with relatively low mean of transformed AUDPS values in each tested year. In contrast, we observed reduced effectiveness of azoxystrobin

(Quadris 2.08SC) with increased transformed AUDPS values over the years of the study (Table 4.11).

There were significant rate effects of the azoxystrobin (Quadris 2.08SC) and cyflufenamid (Torino 0.85SC) on controlling powdery mildew of pumpkin in the field trial in 2017 (Table 4.12). Based on the mean of transformed AUDPS values, we found that the effectiveness of azoxystrobin (Quadris 2.08SC) was significantly ($P = 0.0037$) higher at the rate of 2.00 ml/L and 2.40 ml/L than at 1.70 ml/L. Similarly, the effectiveness of cyflufenamid (Torino 0.85SC) was significantly ($P = 0.0161$) higher at the rate of 0.53 ml/L than at the rates of 0.37 and 0.45 ml/L (Table 4.13). There was no significant rate effect of triflumizole (Procure 480SC), penthiopyrad (Fontelis 1.67SC), and quinoxyfen (Quintec 2.08SC) in the field trial in 2017.

DISCUSSION

Powdery mildew continues to be an important disease of cucurbits in Illinois and other cucurbit producing areas of the US. Although some cucurbit cultivars resistant/tolerant to powdery mildew are available, fungicide application is still an important practice for management of this disease (McGrath, 2001). However, development of resistance in *P. xanthii* to fungicides requires careful use of fungicides and frequent laboratory and field evaluations of their efficacy for management of the disease. Our study provided an in-depth laboratory and field evaluation of effectiveness of major fungicides for the management of cucurbit powdery mildew.

Cucurbit growers in Illinois and other Midwestern states of the US reported severe levels of powdery mildew in cucurbit fields following applications of fungicides recommended for the management of the disease (Babadoost, personal communications). Also, vegetable pathologists

noted losing effectiveness of fungicides for control of powdery mildew in their research plots (McGrath, 2001).

We initiated this study to determine the efficacy of fungicides with different modes of action recommended for the control of powdery mildew. One of the highly effective fungicides for management of powdery mildew is the newly registered fungicide quinoxyfen (Quintec 250SC). Following applications of quinoxyfen in the field, yellowing at the edges of leaves of cucurbits have been observed, which does not cause significant effects on plant growth or yield. However, when we used it on cucumber cotyledons for laboratory assay, it caused chlorosis and death of cotyledons. Therefore, we were not able to generate data on this fungicide in the laboratory part of the study. Additional studies may show the possibility of evaluating the efficacy of quinoxyfen (Quintec 250SC) for control of powdery mildew using cotyledons of other cucurbit species.

We found significant differences among 37 tested isolates of *P. xanthii* to each of the five fungicide groups tested. DMI fungicides have been used for management of cucurbit powdery mildew for more than 20 years (McGrath, 2001). Resistance in *P. xanthii* to some DMI fungicides (e.g., myclobutanil) has been reported. However, myclobutanil (Rally 40W) and triflumizole (Procure 480SC) are widely used for management of *P. xanthii* on cucurbits in Illinois and other Midwestern states (Babadoost, 2010; Egel et al., 2017). We tested triflumizole (Procure 480SC) in the laboratory and in the field, and found that it is still very effective for management of *P. xanthii* on pumpkins. Our results agree with the reports by Babadoost (2016) and Babadoost et al. (2017). This finding indicates that there is likely no serious cross resistance among DMI fungicides. Our findings and reports by Babadoost (2016) and Babadoost et al. (2017) showed that triflumizole (Procure 480SC), and likely other DMI fungicides, can be used

for effectively managing *P. xanthii* on pumpkins, and likely powdery mildew of other cucurbits, in Illinois.

Several investigators have reported resistance in *Podosphaera xanthii* isolates to QoI fungicides (Fernandez-Ortuno et al., 2006; Fernández-Ortuño et al., 2008; McGrath, 2001; McGrath and Shishkoff, 2003; Ishii et al., 2001). Several QoI fungicides, including azoxystrobin (Quadris 2.08SC), pyraclostrobin (Cabrio 20EC), pyraclostrobin + boscalid (Pristine 38WG), and kresoxim-methyl (Sovran 50WG) are used for the management of powdery mildew and other foliage diseases of cucurbits in the Midwestern states (Egel et al., 2017). We evaluated efficacy of azoxystrobin (Quadris 2.08SC) for managing powdery mildew of cucurbits in the laboratory and field settings. Our results showed that the majority of the isolates tested grew well at the concentration of 0.46 μg/ml, compared to the azoxystrobin baseline (0.258 μg/ml) reported in 1998 and 1999 in North America (McGrath and Shishkoff, 2003). In our studies, 29 of 37 isolates showed reduced sensitivity to Quadris 2.08SC (QoI fungicide group). The results from our studies and reports by other investigators (Fernández-Ortuño et al., 2008; McGrath, 2001; McGrath and Shishkoff, 2003; Ishii et al., 2001) indicate that azoxystrobin (Quadris 2.08SC), and likely other QoI fungicides, have lost their effectiveness for managing powdery mildew of cucurbits in the Midwestern states and other cucurbit growing areas in the world.

As a newly introduced fungicide, penthiopyrad (Fontelis 1.67SC), from the SDHI group, was tested for its efficacy for management of *P. xanthii* in cucurbits. The results showed that penthiopyrad provides effective management of powdery mildew of pumpkins. This fungicide is also recommended for management of powdery mildew of cucurbits in the Midwestern states

(Egel et al., 2017). However, additional investigations will be needed to determine the effectiveness of this fungicide for managing powdery mildew of cucurbits in the future.

Quinoxyfen (Quintec 2.08SC) from the quinoline group of fungicides is commonly used for management of cucurbit powdery mildew. Although it has been suggested as a fungicide with low risk for resistance development, a potential risk has been proposed by Davey and McGrath (2006). Our field results showed that quinoxyfen (Quintec 2.08SC) provides effective management of powdery mildew of pumpkin. Also, this fungicide is recommended for management of powdery mildew of cucurbits in the Midwest (Egel et al., 2017).

As another newly introduced fungicide for management of powdery mildew of cucurbits, cyflufenamid (Torino 0.85SC) was tested in this study. The mode of action of this fungicide has yet to be determined. The results of our studies showed that cyflufenamid (Torino 0.85SC) was as effective as triflumizole, penthiopyrad, and quinexyfen for management of powdery mildew of pumpkin. With the existing information, the use of cyflufenamid in cucurbits should be used with caution and in alternation with effective fungicides with different modes of action. Compared to the sensitive isolates from Italy with MICs to cyflufenamid ranging from <0.01 to <1 μ g/ml (from Emilia-Romagna region) and from <10 to >50 μg/ml (from Apulia region) in 2012, our isolates collected in US showed MIC values equal or greater than 0.0498 μg/ml indicating that the isolates from the US are still sensitive to cyflufenamid.

For effective management of cucurbit powdery mildew by application of fungicide, constant monitoring of efficacy of fungicides in different areas will be required. The results of our studies showed that field testing is reliable for assessing the effectiveness of fungicides for managing cucurbit powdery mildew. However, field testing in different locations of the state would be needed to provide more reliable assessment of the fungicide efficacy.

Table 4.1. Fungicides tested in the laboratory and field for their efficacy in controlling powdery mildew of cucurbits caused by *Podosphaera xanthii*.

Location of test	Fungicide name		Fungicide group	FRAC[z] code	Year tested
	Common name	Trade name			
Laboratory	Triflumizole	Procure 480SC	DMI	3	2015, 2016
	Azoxystrobin	Quadris 2.08SC	QoI	11	2015, 2016
	Penthiopyrad	Fontelis 1.67SC	SDHI	7	2015, 2016
	Cyflufenamid	Torino 0.85SC	Unknown	U[y]6	2015, 2016
Field	Triflumizole	Procure 480SC	DMI	3	2013, 2014, 2015, 2016, 2017
	Azoxystrobin	Quadris 2.08SC	QoI	11	2011, 2012, 2015, 2017
	Penthiopyrad	Fontelis 1.67SC	SDHI	7	2012, 2013, 2015, 2016, 2017
	Quinoxyfen	Quintec 250SC	Quinolines	13	2013, 2014, 2015, 2016, 2017
	Cyflufenamid	Torino 0.85SC	Unknown	U6	2015, 2016, 2017

[z] FRAC = fungicide resistance action committee. FRAC codes indicate the mode of actions of fungicides. (http://ipm.ifas.ufl.edu/resources/success_stories/T&PGuide/pdfs/Appendices/Appendix6- FRAC.pdf).

[y] Unknown mode of action.

Table 4.2. Minimal inhibitory concentration (MIC) of fungicides for preventing infection of cotyledons of cucumber 'Bush Crops' by *Podosphaera xanthii* isolates in the laboratory studies.

Isolate	Year	Location		Host species	MIC			
		State[z]	County		Procure (DMI)[y]	Quadris (QoI)[x]	Fontelis (SDHI)[w]	Torino (U6)[v]
IL04	2015	IL	Champaign	*Cucumis melo*	0.81	0.46	20.41	0.0498
IL05	2015	IL	Kane	*Cucumis sativus*	0.81	>342.46	4.08	0.0498
IL06	2015	IL	Champaign	*Cucumis sativus*	4.04	>342.46	20.41	0.0498
IL08	2015	IL	McHenry	*Cucurbita pepo*	4.04	>342.46	4.08	>0.0498
IL09	2015	IL	McHenry	*Cucurbita pepo*	4.04	>342.46	20.41	0.0498
IL10	2015	IL	Calhoun	*Cucurbita pepo*	4.04	>342.46	20.41	0.0498
IL11	2015	IL	Calhoun	*Cucurbita pepo*	4.04	>342.46	20.41	0.0498
IL14	2015	IL	Calhoun	*Cucurbita pepo*	0.81	0.46	20.41	0.0498
IL17	2015	IL	Champaign	*Cucurbita pepo*	0.81	45.66	20.41	>0.0498
IL18	2015	IL	Champaign	*Cucurbita pepo*	4.04	>342.46	4.08	0.0498
IL19	2015	IL	Champaign	*Cucurbita pepo*	0.81	>342.46	4.08	0.0498
IL20	2015	IL	Tazewell	*Cucurbita moschata*	4.04	>342.46	4.08	0.0498
IL21	2015	IL	Tazewell	*Cucurbita moschata*	4.04	>342.46	4.08	0.0498
IL22	2015	IL	Champaign	*Cucurbita moschata*	0.81	>0.46	20.41	>0.0498
IL23	2015	IL	Champaign	*Cucurbita moschata*	0.81	0.46	20.41	0.0498
IL24	2015	IL	Kane	*Cucurbita pepo*	4.04	>342.46	20.41	0.0498
IL28	2015	IL	Douglas	*Cucurbita pepo*	0.81	>342.46	4.08	0.0498
IL29	2015	IL	McHenry	*Cucurbita pepo*	4.04	>342.46	4.08	>0.0498
IL30	2015	IL	Champaign	*Cucurbita pepo*	4.04	>342.46	20.41	0.0498
IL33	2015	IL	Douglas	*Cucurbita pepo*	4.04	>342.46	4.08	0.0498
CA8-1	2016	CA	Monterey	Unknown	>4.04	>342.46	>20.41	>0.0498
CA9-1	2016	CA	San Benito	*Cucurbita maxima*	>4.04	>342.46	>20.41	>0.0498
IA1-2	2016	IA	Muscatine	*Cucurbita pepo*	>4.04	>342.46	>20.41	>0.0498
IL5	2016	IL	Wayne	*Cucurbita pepo*	>4.04	>342.46	>20.41	>0.0498
IL7	2016	IL	Tazewell	*Cucurbita pepo*	>4.04	>342.46	>20.41	0.0498
ILD1	2016	IL	Macon	*Cucumis melo*	0.81	45.66	>20.41	>0.0498

Table 4.2 (cont.)

ILD2	2016	IL	Macon	*Cucumis melo*	>4.04	>342.46	>20.41	>0.0498
ILN2	2016	IL	DuPage	*Cucumis sativus*	>4.04	>342.46	>20.41	0.0498
ILN3-2	2016	IL	DuPage	*Cucurbita pepo*	>4.04	>342.46	>20.41	0.0498
ILV1-1	2016	IL	Fayette	*Cucurbita moschata*	0.08	45.66	45.66	>20.41
ILV4-2	2016	IL	Fayette	*Cucurbita pepo*	>4.04	45.66	45.66	>20.41
IN3-1	2016	IN	Hancock	*Cucurbita pepo*	>4.04	>342.46	>342.46	>20.41
IN3-2	2016	IN	Hancock	*Cucurbita pepo*	4.04	>342.46	>342.46	>20.41
MI3-1	2016	MI	Berrien	*Cucurbita pepo*	>4.04	>342.46	>342.46	>20.41
MI6-1	2016	MI	Berrien	*Cucurbita pepo*	>4.04	>342.46	>342.46	>20.41
TX1-2	2016	TX	Potter	*Cucurbita pepo*	>4.04	>342.46	>342.46	>20.41
TX3-2	2016	TX	Potter	*Cucumis sativus*	>4.04	>342.46	>342.46	>20.41

[z] CA = California, IL = Illinois, IN = Indiana, MI = Michigan, TX = Texas.
[y] Triflumizole (Procure 480SC; Chemtura, Middlebury, CT).
[x] Azoxystrobin (Quadris 2.08SC; Syngenta, Greensboro, NC).
[w] Penthiopyrad (Fontelis 1.67SC; DuPont, Wilmington, DE).
[v] Cyflufenamid (Torino 0.85SC; Gowan, Yuma, AZ).

Table 4.3. Analysis of variance for the infected area (cm^2) of cotyledons of cucumber 'Bush Crop' by 37 isolates of *Podosphaera xanthii* following applications of fungicides.

Fungicide	Term	DF[z]	Sum square	Mean square	F value	P. value
Triflumizole (Procure 480SC)	Conc.[y]	1	60.12	60.12	1087.21	<0.0001*
	Isolate	36	30.13	0.84	15.14	<0.0001*
	Conc.:Isolate	36	9.89	0.27	4.97	<0.0001*
	Residuals	1276	70.55	0.06		
Azoxystrobin (Quadris 2.08SC)	Conc.	1	45.95	45.95	575.11	<0.0001*
	Isolate	36	34.17	0.95	11.88	<0.0001*
	Conc.:Isolate	36	7.49	0.21	2.60	<0.0001*
	Residuals	901	71.99	0.08		
Penthiopyrad (Fontelis 1.67SC)	Conc.	1	20.97	20.97	262.53	<0.0001*
	Isolate	36	52.01	1.44	18.08	<0.0001*
	Conc.:Isolate	36	7.71	0.21	2.68	<0.0001*
	Residuals	953	76.13	0.08		
Cyflufenamid (Torino 0.85sc) 2015	Conc.	1	27.62	27.62	861.64	<0.0001*
	Isolate	19	3.03	0.16	4.98	<0.0001*
	Conc.:Isolate	19	1.02	0.05	1.68	0.0368*
	Residuals	383	12.28	0.03		
Cyflufenamid (Torino 0.85sc) 2016	Conc.	1	33.08	33.08	750.60	<0.0001*
	Isolate	16	5.32	0.33	7.55	<0.0001*
	Conc.:Isolate	16	4.57	0.29	6.48	<0.0001*
	Residuals	564	24.86	0.04		

[z] Degree of freedom.
[y] Concentration.

Table 4.4. Least squared means (LSM) of the cotyledon infection area (IA) (cm^2) by isolates of *Podosphaera xanthii* following application of triflumizole (Procure 480SC) fungicide.

	Fungicide concentration								
	0.08 µg/ml			0.81 µg/ml			4.04 µg/ml		
Isolate	**LSM**	**SE**	***P.* value**	**LSM**	**SE**	***P.* value**	**LSM**	**SE**	***P.* value[z]**
IA1-2	0.92	0.05	<0.0001	0.89	0.04	<0.0001	0.77	0.08	<0.0001 a
TX3-2	0.92	0.05	<0.0001	0.88	0.04	<0.0001	0.72	0.08	<0.0001 ab
ILN2	0.79	0.05	<0.0001	0.77	0.04	<0.0001	0.68	0.08	<0.0001 a-c
MI6-1	0.82	0.05	<0.0001	0.79	0.04	<0.0001	0.62	0.08	<0.0001 a-c
ILD2	0.66	0.05	<0.0001	0.65	0.04	<0.0001	0.57	0.08	<0.0001 a-d
CA9-1	0.69	0.05	<0.0001	0.66	0.04	<0.0001	0.55	0.08	<0.0001 a-e
IN3-1	0.80	0.05	<0.0001	0.73	0.04	<0.0001	0.42	0.08	<0.0001 a-f
IL5	0.70	0.05	<0.0001	0.65	0.04	<0.0001	0.42	0.08	<0.0001 a-f
ILN3-2	0.91	0.05	<0.0001	0.81	0.04	<0.0001	0.39	0.08	<0.0001 a-g
IL7	0.68	0.05	<0.0001	0.62	0.04	<0.0001	0.35	0.08	<0.0001 a-h
MI3-1	0.81	0.05	<0.0001	0.73	0.04	<0.0001	0.33	0.08	<0.0001 a-i
ILV4-2	0.60	0.05	<0.0001	0.55	0.04	<0.0001	0.31	0.08	0.0001 b-j
CA8-1	0.79	0.05	<0.0001	0.69	0.04	<0.0001	0.29	0.08	0.0005 b-j
TX1-2	0.83	0.05	<0.0001	0.73	0.04	<0.0001	0.26	0.08	0.0016 c-j
IL20	0.81	0.05	<0.0001	0.66	0.04	<0.0001	0.03	0.11	0.7702 d-j
IL08	0.79	0.04	<0.0001	0.65	0.04	<0.0001	0.03	0.08	0.7303 f-j
IL29	0.73	0.05	<0.0001	0.60	0.04	<0.0001	0.02	0.08	0.8473 f-j
IL06	0.78	0.06	<0.0001	0.64	0.05	<0.0001	0.01	0.11	0.9049 e-j
IL21	0.79	0.05	<0.0001	0.65	0.04	<0.0001	0.01	0.08	0.9311 f-j
IL30	0.70	0.04	<0.0001	0.57	0.04	<0.0001	-0.01	0.08	0.8939 f-j
IL33	0.70	0.05	<0.0001	0.57	0.04	<0.0001	-0.02	0.08	0.8349 f-j
IL18	0.73	0.05	<0.0001	0.59	0.04	<0.0001	-0.03	0.08	0.7176 g-j
IL09	0.72	0.05	<0.0001	0.58	0.04	<0.0001	-0.03	0.08	0.7050 g-j
IL24	0.67	0.04	<0.0001	0.54	0.04	<0.0001	-0.04	0.08	0.6633 g-j
IL10	0.73	0.04	<0.0001	0.59	0.04	<0.0001	-0.04	0.08	0.6295 g-j
IL11	0.63	0.04	<0.0001	0.50	0.04	<0.0001	-0.05	0.08	0.5320 g-j
IL04	0.52	0.05	<0.0001	0.42	0.04	<0.0001	-0.05	0.08	0.5004 g-j
IL28	0.59	0.04	<0.0001	0.47	0.04	<0.0001	-0.06	0.08	0.4555 h-j
IL17	0.63	0.05	<0.0001	0.50	0.04	<0.0001	-0.06	0.08	0.4356 h-j
IN3-2	0.46	0.05	<0.0001	0.36	0.04	<0.0001	-0.07	0.08	0.3785 h-j
IL14	0.48	0.04	<0.0001	0.37	0.04	<0.0001	-0.08	0.08	0.3223 h-j
IL05	0.51	0.05	<0.0001	0.40	0.04	<0.0001	-0.08	0.08	0.3225 h-j
ILV1-1	0.50	0.05	<0.0001	0.40	0.04	<0.0001	-0.08	0.08	0.3171 h-j
IL22	0.54	0.05	<0.0001	0.42	0.04	<0.0001	-0.08	0.08	0.2989 h-j
IL23	0.51	0.05	<0.0001	0.40	0.04	<0.0001	-0.09	0.08	0.2769 h-j

Table 4.4 (cont.)

ILD1	0.64	0.05	<0.0001	0.50	0.04	<0.0001	-0.10	0.08	0.2382 ij
IL19	0.55	0.05	<0.0001	0.43	0.04	<0.0001	-0.12	0.08	0.1442 j

[z] Means followed by the same letter are not significantly different according to Tukey's significant difference test (alpha = 0.05).

Table 4.5. Least squared means (LSM) of the cotyledon infection area (IA) (cm^2) by isolates of *Podosphaera xanthii* following application of azoxystrobin (Quadris 2.08SC) fungicide.

	Fungicide concentration								
	0.46 µg/ml			45.66 µg/ml			342.46 µg/ml		
Isolate	**LSM**	**SE**	***P.* value**	**LSM**	**SE**	***P.* value**	**LSM**	**SE**	***P.* value[z]**
TX3-2	0.89	0.06	<0.0001	0.88	0.06	<0.0001	0.76	0.11	<0.0001 ab
IA1-2	0.86	0.07	<0.0001	0.84	0.06	<0.0001	0.74	0.10	<0.0001 a
IL5	0.76	0.07	<0.0001	0.75	0.06	<0.0001	0.68	0.11	<0.0001 a-c
ILD2	0.85	0.07	<0.0001	0.82	0.06	<0.0001	0.61	0.10	<0.0001 a-d
TX1-2	0.85	0.06	<0.0001	0.81	0.05	<0.0001	0.58	0.10	<0.0001 a-d
MI3-1	0.89	0.06	<0.0001	0.85	0.05	<0.0001	0.56	0.10	<0.0001 a-d
IN3-1	0.91	0.06	<0.0001	0.85	0.06	<0.0001	0.49	0.10	<0.0001 a-d
CA8-1	0.76	0.06	<0.0001	0.72	0.06	<0.0001	0.49	0.10	<0.0001 a-d
ILN3-2	0.99	0.06	<0.0001	0.92	0.05	<0.0001	0.48	0.11	<0.0001 a-e
MI6-1	0.82	0.06	<0.0001	0.78	0.05	<0.0001	0.48	0.10	<0.0001 a-e
CA9-1	0.69	0.07	<0.0001	0.66	0.06	<0.0001	0.41	0.10	<0.0001 a-e
IL08	0.72	0.07	<0.0001	0.67	0.06	<0.0001	0.35	0.11	0.00090 a-f
IL18	1.06	0.07	<0.0001	0.96	0.07	<0.0001	0.26	0.11	0.01493 a-f
IL7	0.60	0.07	<0.0001	0.55	0.06	<0.0001	0.25	0.10	0.01097 a-f
IL06	0.88	0.07	<0.0001	0.80	0.06	<0.0001	0.22	0.11	0.03958 a-f
IL28	0.92	0.07	<0.0001	0.83	0.06	<0.0001	0.22	0.11	0.03931 a-f
IL19	0.85	0.07	<0.0001	0.77	0.06	<0.0001	0.22	0.11	0.04124 a-f
IL24	0.83	0.07	<0.0001	0.75	0.06	<0.0001	0.21	0.11	0.04365 a-f
IL30	0.87	0.07	<0.0001	0.78	0.07	<0.0001	0.20	0.11	0.05955 a-f
IL33	0.83	0.07	<0.0001	0.74	0.06	<0.0001	0.19	0.11	0.07049 a-f
IL10	0.86	0.07	<0.0001	0.77	0.07	<0.0001	0.19	0.11	0.07113 a-f
IL20	0.77	0.07	<0.0001	0.70	0.06	<0.0001	0.18	0.11	0.09165 b-f
IL07	0.83	0.07	<0.0001	0.74	0.07	<0.0001	0.18	0.11	0.09321 b-f
IL05	0.76	0.07	<0.0001	0.68	0.06	<0.0001	0.17	0.11	0.11814 c-f
IL11	0.86	0.07	<0.0001	0.77	0.06	<0.0001	0.16	0.11	0.13134 c-f
IL21	0.67	0.07	<0.0001	0.60	0.07	<0.0001	0.15	0.11	0.15861 c-f
IL29	0.68	0.08	<0.0001	0.61	0.07	<0.0001	0.15	0.11	0.17021 c-f
ILN2	0.77	0.06	<0.0001	0.69	0.05	<0.0001	0.14	0.10	0.15747 c-f
IL17	0.71	0.07	<0.0001	0.64	0.06	<0.0001	0.13	0.11	0.21111 c-f
IL22	0.40	0.07	<0.0001	0.36	0.06	<0.0001	0.07	0.11	0.49122 d-f
IL04	0.36	0.07	<0.0001	0.32	0.06	<0.0001	0.07	0.11	0.53628 d-f
IL23	0.33	0.07	<0.0001	0.29	0.06	<0.0001	0.06	0.11	0.57016 d-f
IL14	0.31	0.07	<0.0001	0.28	0.06	<0.0001	0.06	0.11	0.59383 d-f
IN3-2	0.71	0.07	<0.0001	0.61	0.06	<0.0001	-0.05	0.10	0.59085 ef
ILV4-2	0.47	0.06	<0.0001	0.39	0.05	<0.0001	-0.14	0.10	0.15107 f

Table 4.5 (cont.)

ILD1	0.48	0.06	<0.0001	0.40	0.05	<0.0001	-0.14	0.10	0.14185 f
ILV1-1	0.55	0.06	<0.0001	0.46	0.05	<0.0001	-0.16	0.10	0.09397 f

[z] Means followed by the same letter are not significantly different according to Tukey's significant difference test (alpha = 0.05).

Table 4.6. Least squared means (LSM) of the cotyledon infection area (IA) (cm^2) by isolates of *Podosphaera xanthii* following application of penthiopyrad (Fontelis 1.67SC) fungicide.

Isolate	Fungicide concentration								
	0.41 µg/ml			4.08 µg/ml			20.41 µg/ml		
	LSM	SE	*P*. value	LSM	SE	*P*. value	LSM	SE	*P*. value[z]
ILN3-2	0.98	0.04	<0.0001	0.97	0.04	<0.0001	0.93	0.07	<0.0001 a
MI3-1	0.96	0.04	<0.0001	0.95	0.04	<0.0001	0.90	0.07	<0.0001 a
ILD1	0.91	0.04	<0.0001	0.91	0.04	<0.0001	0.88	0.07	<0.0001 a
TX3-2	0.96	0.04	<0.0001	0.94	0.04	<0.0001	0.87	0.07	<0.0001 ab
ILD2	0.88	0.04	<0.0001	0.87	0.04	<0.0001	0.83	0.07	<0.0001 a-c
ILN2	0.87	0.04	<0.0001	0.86	0.04	<0.0001	0.80	0.07	<0.0001 a-d
MI6-1	0.88	0.04	<0.0001	0.86	0.04	<0.0001	0.78	0.07	<0.0001 a-d
IN3-1	0.92	0.04	<0.0001	0.89	0.04	<0.0001	0.73	0.07	<0.0001 a-d
CA9-1	0.88	0.04	<0.0001	0.85	0.04	<0.0001	0.73	0.07	<0.0001 a-d
IA1-2	0.99	0.04	<0.0001	0.94	0.04	<0.0001	0.72	0.07	<0.0001 a-d
TX1-2	0.89	0.04	<0.0001	0.84	0.04	<0.0001	0.64	0.07	<0.0001 a-e
IN3-2	0.76	0.04	<0.0001	0.73	0.04	<0.0001	0.59	0.07	<0.0001 a-e
CA8-1	0.83	0.04	<0.0001	0.78	0.04	<0.0001	0.57	0.07	<0.0001 a-e
IL5	0.75	0.04	<0.0001	0.70	0.04	<0.0001	0.48	0.07	<0.0001 b-f
ILV1-1	0.97	0.04	<0.0001	0.88	0.04	<0.0001	0.46	0.07	<0.0001 c-f
ILV4-2	0.76	0.04	<0.0001	0.70	0.04	<0.0001	0.43	0.07	<0.0001 d-g
IL7	0.67	0.04	<0.0001	0.60	0.04	<0.0001	0.28	0.07	<0.0001 e-h
IL30	0.74	0.05	<0.0001	0.61	0.04	<0.0001	0.02	0.10	0.8357 f-h
IL14	0.94	0.08	<0.0001	0.77	0.07	<0.0001	0.02	0.10	0.8532 f-h
IL23	0.82	0.05	<0.0001	0.67	0.04	<0.0001	0.02	0.10	0.8663 f-h
IL17	0.87	0.05	<0.0001	0.71	0.04	<0.0001	0.01	0.10	0.9188 f-h
IL10	0.76	0.05	<0.0001	0.62	0.04	<0.0001	0.01	0.10	0.9334 f-h
IL11	0.80	0.05	<0.0001	0.66	0.04	<0.0001	0.00	0.10	0.9618 f-h
IL24	0.75	0.05	<0.0001	0.61	0.04	<0.0001	0.00	0.10	0.9930 f-h
IL06	0.87	0.08	<0.0001	0.71	0.07	<0.0001	-0.01	0.10	0.9327 f-h
IL09	0.73	0.05	<0.0001	0.59	0.04	<0.0001	-0.03	0.10	0.7466 gh
IL22	0.73	0.08	<0.0001	0.59	0.07	<0.0001	-0.04	0.10	0.6813 gh
IL04	0.76	0.05	<0.0001	0.61	0.04	<0.0001	-0.05	0.10	0.5966 h
IL20	0.65	0.05	<0.0001	0.52	0.04	<0.0001	-0.06	0.10	0.5291 h
IL18	0.54	0.08	<0.0001	0.42	0.07	<0.0001	-0.08	0.10	0.4332 h
IL21	0.58	0.08	<0.0001	0.45	0.07	<0.0001	-0.09	0.10	0.3976 h
IL29	0.56	0.05	<0.0001	0.44	0.04	<0.0001	-0.09	0.10	0.3642 h
IL19	0.57	0.05	<0.0001	0.44	0.04	<0.0001	-0.11	0.10	0.2717 h
IL08	0.57	0.05	<0.0001	0.44	0.04	<0.0001	-0.11	0.10	0.2664 h
IL05	0.56	0.05	<0.0001	0.44	0.04	<0.0001	-0.12	0.10	0.2250 h

Table 4.6 (cont.)

IL33	0.48	0.05	<0.0001	0.37	0.04	<0.0001	-0.12	0.10	0.2213 h
IL28	0.50	0.05	<0.0001	0.38	0.04	<0.0001	-0.14	0.10	0.1544 h

[z] Means followed by the same letter are not significantly different according to Tukey's significant difference test (alpha = 0.05).

Table 4.7. Least squared means (LSM) of the cotyledon infection area (IA) (cm^2) by isolates of *Podosphaera xanthii* following application of cyflufenamid (Torino 0.85SC) fungicide.

		Fungicide concentration								
		0.0010 µg/ml			0.0100 µg/ml			0.0498 µg/ml		
Year	**Isolate**	**LSM**	**SE**	***P*. value**	**LSM**	**SE**	***P*. value**	**LSM**	**SE**	***P*. value[z]**
2015	IL22	0.72	0.04	<0.0001	0.65	0.04	<0.0001	0.32	0.09	0.0003 a
	IL17	0.90	0.04	<0.0001	0.79	0.04	<0.0001	0.31	0.06	<0.0001 a
	IL29	0.74	0.05	<0.0001	0.66	0.04	<0.0001	0.29	0.12	0.0177 a
	IL08	0.87	0.04	<0.0001	0.71	0.04	<0.0001	0.04	0.06	0.5035 a
	IL24	0.71	0.04	<0.0001	0.58	0.04	<0.0001	0.02	0.09	0.8154 a
	IL09	0.81	0.04	<0.0001	0.66	0.04	<0.0001	0.02	0.09	0.8451 a
	IL05	0.74	0.05	<0.0001	0.60	0.04	<0.0001	0.02	0.09	0.8503 a
	IL14	0.89	0.04	<0.0001	0.73	0.04	<0.0001	0.00	0.09	0.9738 a
	IL06	0.80	0.05	<0.0001	0.66	0.04	<0.0001	0.00	0.09	0.9930 a
	IL20	0.85	0.05	<0.0001	0.69	0.04	<0.0001	0.00	0.12	0.9834 a
	IL33	0.69	0.04	<0.0001	0.56	0.04	<0.0001	0.00	0.09	0.9557 a
	IL18	0.77	0.04	<0.0001	0.63	0.04	<0.0001	-0.01	0.09	0.9419 a
	IL11	0.81	0.04	<0.0001	0.66	0.04	<0.0001	-0.01	0.09	0.8912 a
	IL04	0.81	0.04	<0.0001	0.66	0.04	<0.0001	-0.01	0.09	0.8835 a
	IL19	0.78	0.04	<0.0001	0.63	0.04	<0.0001	-0.03	0.09	0.7105 a
	IL10	0.89	0.04	<0.0001	0.72	0.04	<0.0001	-0.03	0.09	0.7008 a
	IL23	0.61	0.04	<0.0001	0.48	0.04	<0.0001	-0.11	0.12	0.3769 a
	IL21	0.75	0.07	<0.0001	0.57	0.09	<0.0001	-0.24	0.57	0.6766 a
	IL30	0.61	0.06	<0.0001	0.38	0.09	<0.0001	-0.66	0.54	0.2187 a
	IL28	0.57	0.06	<0.0001	0.26	0.13	0.0429	-1.14	0.71	0.1069 a
2016	MI3-1	0.88	0.04	<0.0001	0.86	0.04	<0.0001	0.76	0.07	<0.0001 a
	IN3-1	0.84	0.04	<0.0001	0.81	0.04	<0.0001	0.64	0.07	<0.0001 a
	ILD2	0.76	0.04	<0.0001	0.72	0.04	<0.0001	0.51	0.08	<0.0001 ab
	TX3-2	0.91	0.04	<0.0001	0.79	0.04	<0.0001	0.25	0.07	0.0006 bc
	IL5	0.69	0.04	<0.0001	0.61	0.04	<0.0001	0.22	0.07	0.0023 bc
	ILN3-2	0.97	0.04	<0.0001	0.83	0.04	<0.0001	0.22	0.07	0.0024 bc
	TX1-2	0.85	0.04	<0.0001	0.73	0.04	<0.0001	0.22	0.07	0.0025 bc
	IA1-2	0.81	0.04	<0.0001	0.70	0.04	<0.0001	0.20	0.07	0.0064 bc
	CA9-1	0.78	0.04	<0.0001	0.67	0.04	<0.0001	0.19	0.07	0.0086 bc
	ILD1	0.87	0.04	<0.0001	0.74	0.04	<0.0001	0.17	0.07	0.0179 bc
	ILN2	0.84	0.04	<0.0001	0.72	0.04	<0.0001	0.17	0.07	0.0229 bc
	ILV4-2	0.72	0.04	<0.0001	0.61	0.04	<0.0001	0.14	0.08	0.0936 bc
	CA8-1	0.78	0.04	<0.0001	0.66	0.04	<0.0001	0.13	0.07	0.0843 c
	ILV1-1	1.04	0.04	<0.0001	0.86	0.04	<0.0001	0.06	0.07	0.4371 c

Table 4.7 (cont.)

IL7	0.65	0.04	<0.0001	0.53	0.04	<0.0001	0.03	0.08	0.7034 c
IN3-2	0.83	0.04	<0.0001	0.68	0.04	<0.0001	0.01	0.08	0.9094 c
MI6-1	0.76	0.04	<0.0001	0.62	0.04	<0.0001	-0.01	0.07	0.9331 c

[z] Means followed by the same letter are not significantly different according to Tukey's significant difference test (alpha = 0.05).

Table 4.8. Frequency[z] of *Podosphaera xanthii* isolates classified in different sensitivity groups to the fungicides.

Fungicide	MIC[y] group	Location				Total Freq.
		Northern Illinois[x]	Central Illinois[w]	Southern Illinois[v]	Other States[u]	
Triflumizole (Procure 480SC)[t]	Less sensitive	7/8	8/15	3/5	9/9	27/37
	More sensitive	1/8	7/15	2/5	0/9	10/37
Azoxystrobin (Quadris 2.08SC)[s]	Less sensitive	8/8	10/15	2/5	9/9	29/37
	More sensitive	0/8	5/15	3/5	0/9	8/37
Penthiopyrad (Fontelis 1.67SC)[r]	Less sensitive	5/8	9/15	5/5	9/9	28/37
	More sensitive	3/8	6/15	0/5	0/9	9/37
Cyflufenamid (Torino 0.85SC)[p]	-	8/8	15/15	5/5	9/9	37/37
	Total	8	15	5	9	37

[z] Frequency of sensitivity represent number of isolates in the sensitivity group.
[y] MIC = minimum inhibitory concentrations.
[x] Isolates collected from Kane, McHenry, and Will County in northern Illinois.
[w] Isolates collected from Champaign, Douglas, Macon, and Tazewell County in central Illinois.
[v] Isolates collected from Calhoun, Fayette, and Wayne County in southern Illinois.
[u] Isolates collected from California, Iowa, Indiana, Michigan, and Texas.
[t] The isolates with MIC ≥ 4.04 µg/ml were grouped in the less sensitive group, and the isolates with MIC from 0.08 to 0.81 µg/ml were grouped in the more sensitive group for DMI fungicide triflumizole.
[s] The isolates with MIC ≥ 342.46 µg/ml were grouped in the less sensitive group, and the isolates with MIC from 0.46 to 45.66 µg/ml were grouped in the more sensitive group for QoI fungicide azoxystrobin.
[r] The isolates with MIC ≥ 20.41 µg/ml were grouped in the less sensitive group, and the isolates with MIC 4.08 µg/ml were grouped in the more sensitive group for SDHI fungicide penthiopyrad.
[p] All the isolates were found with MIC ≥ 0.0498 µg/ml.

Table 4.9. Schedules of sowing seeds, applications of fungicides, and assessing disease severity for fungicide trials on jack-o-lantern pumpkin 'Howden' at the University of Illinois Vegetable Research Farm in Champaign, IL during 2011-2017.

Year	Action	Dates of the actions
2011	Seed sowing	9 June
	Fungicide application	19 July, 26 July, 2 August, 9 August, 16 August, 23 August, 30 August
	Assessing disease severity	2 August, 9 August, 16 August, 23 August, 30 August, 6 September
2012	Seed sowing	4 June
	Fungicide application	16 July, 23 July, 30 July, 6 August, 13 August, 20 August, 27 August
	Assessing disease severity	10 August, 24 August, 7 September, 13 September
2013	Seed sowing	4 June
	Fungicide application	19 July, 26 July, 2 August, 9 August, 16 August, 23 August, 30 August
	Assessing disease severity	3 August, 7 August, 31 August, 14 September
2014	Seed sowing	16 June
	Fungicide application	25 July, 1 August, 8 August, 15 August, 22 August, 29 August, 5 September
	Assessing disease severity	6 August, 20 August, 3 September, 17 September
2015	Transplanting [z] seedlings	30 June
	Fungicide application	30 July, 6 August, 20 August, 19 August, 27 August, 3 September, 10 September
	Assessing disease severity	31 July, 10 August, 20 Aug, 29 Aug, 6 September, 14 September
2016	Seed sowing	9 June
	Fungicide application	14 July, 21 July, 28 July, 4 August, 11 August, 25 August, 1 September
	Assessing disease severity	3 August, 17 August, 31 August, 14 September
2017	Seed sowing	4 June
	Fungicide application	10 July, 17 July, 24 July, 31 July, 7 August, 14 August, 21 August, 28 August
	Assessing disease severity	29 July, 6 August, 13 August, 20 August, 27 August, 4 September

[z] In 2015, Because of heavy rain falls and flooding in 2015 some of plots from seeding were lost, therefore a new trial was set up with transplants.

Table 4.10. Analysis of variance for the transformed area under the disease progress stairs (AUDPS) for fungicides tested in the field[z].

Treatment	Term	DF	Sum square	Mean square	F value	P value
Triflumizole (Procure 480SC)	Fungicide	1	73.11	73.11	887.11	<0.0001*
	Year	1	0.19	0.19	2.36	0.1500
	Fungicide:Year	1	0	0	0.05	0.8332
	Residuals	12	0.99	0.08		
Azoxystrobin (Quadris 2.08SC)	Fungicide	1	52.75	52.75	347.65	<0.0001*
	Year	3	13.94	4.65	30.63	<0.0001*
	Fungicide:Year	3	13.37	4.46	29.36	<0.0001*
	Residuals	24	3.64	0.15		
Penthiopyrad (Fontelis 1.67SC)	Fungicide	1	106.27	106.27	342.06	<0.0001*
	Year	4	19.97	4.99	16.07	<0.0001*
	Fungicide:Year	4	11.04	2.76	8.88	0.0001
	Residuals	30	9.32	0.31		
Quinoxyfen (Quintec 2.08SC)	Fungicide	1	61.17	61.17	361.8	<0.0001*
	Year	1	1.94	1.94	11.45	0.0054
	Fungicide:Year	1	1.02	1.02	6.05	0.0300
	Residuals	12	2.03	0.17		
Cyflufenamid (Torino 0.85SC)	Fungicide	1	66.85	66.85	201.41	<0.0001*
	Year	2	0.77	0.38	1.16	0.3369
	Fungicide:Year	2	2.97	1.48	4.47	0.0265
	Residuals	18	5.97	0.33		

[z] Efficacy of fungicides were based on comparison with untreated control plots.

Table 4.11. The transformed area under powdery mildew disease progress stairs (AUDPS) in pumpkin plots treated with different fungicides during 2011-2017[z].

Fungicide	Treatment	AUDPS
Triflumizole (Procure 480SC)	2015:control	6.80 a[y]
	2017:control	6.61 a
	2015:triflumizole	2.55 b
	2017:triflumizole	2.30 b
Azoxystrobin (Quadris 2.08SC)	2011:control	6.90 a
	2015:control	6.80 a
	2012:control	6.64 a
	2017:control	6.61 a
	2015: azoxystrobin	5.64 b
	2017: azoxystrobin	5.09 b
	2011: azoxystrobin	3.64 c
	2012: azoxystrobin	2.30 d
Penthiopyrad (Fontelis 1.67SC)	2016:control	7.29 a
	2011:control	6.90 a
	2015:control	6.80 a
	2012:control	6.64 a
	2017:control	6.61 a
	2016:penthiopyrad	5.59 b
	2015:penthiopyrad	4.34 c
	2017:penthiopyrad	3.13 d
	2012:penthiopyrad	2.52 d
	2011:penthiopyrad	2.35 d
Quinoxyfen (Quintec 2.08SC)	2015:control	6.80 a
	2017:control	6.61 a
	2015:quinoxyfen	3.39 b
	2017:quinoxyfen	2.19 c
Cyflufenamid (Torino 0.85SC)	2016:control	7.29 a
	2015:control	6.80 a
	2017:control	6.61 a
	2015:cyflufenamid	4.16 b
	2017:cyflufenamid	3.53 bc
	2016:cyflufenamid	2.99 c

[z] Trials were conducted on pumpkin 'Howden' at the University of Illinois Vegetable Farm in Champaign, Illinois.
[y] For each fungicide, means followed by the same letter are not significantly different according to the Fisher's LSD ($P = 0.05$).

Table 4.12. Analysis of variance of efficacy of different rates of fungicides used in the control of powdery mildew (infected by *Podosphaera xanthii*) on pumpkin 'Howden' in the field in 2017.

Fungicide	Term	DF	Sum square	Mean square	F value	P value
Triflumizole (Procure 480SC)	Fungicide	1	107.47	107.47	3818.27	<0.0001*
	Rate	1	0.00	0.00	0.00	1.0000
	Residuals	21	0.59	0.03		
Azoxystrobin (Quadris 2.08SC)	Fungicide	1	6.68	6.68	47.10	<0.0001*
	Rate	1	1.52	1.52	10.69	0.0037
	Residuals	21	2.98	0.14		
Penthiopyrad (Fontelis 1.67SC)	Fungicide	1	55.82	55.82	162.08	<0.0001*
	Rate	1	0.73	0.73	2.12	0.1599
	Residuals	21	7.23	0.34		
Quinoxyfen (Quintec 2.08SC)	Fungicide	1	128.08	128.08	409.88	<0.0001*
	Rate	1	0.08	0.08	0.26	0.6169
	Residuals	21	6.56	0.31		
Cyflufenamid (Torino 0.85SC)	Fungicide	1	32.59	32.59	113.09	<0.0001*
	Rate	1	1.98	1.98	6.86	0.0161
	Residuals	21	6.05	0.29		

Table 4.13. The transformed area under disease progress stairs (AUDPS) of powdery mildew on pumpkin 'Howden' (caused by *Podosphaera xanthii*) following applications of different rates of fungicides in the field in 2017.

Fungicides	**Rate (ml/L)**	**AUDPS**
Triflumizole (Procure 480SC)	0.00	6.53 a[z]
	0.62	2.30 b
	0.93	2.30 b
	1.24	2.30 b
Azoxystrobin (Quadris 2.08SC)	0.00	6.53 a
	1.70	5.98 b
	2.00	5.37 c
	2.40	5.09 c
Penthiopyrad (Fontelis 1.67SC)	0.00	6.53 a
	1.90	3.74 b
	2.20	3.58 b
	2.50	3.13 b
Quinoxyfen (Quintec 2.08SC)	0.00	6.53 a
	0.93	2.19 b
	0.62	1.98 b
	0.78	1.57 b
Cyflufenamid (Torino 0.85SC)	0.00	6.53 a
	0.45	4.55 b
	0.37	4.53 b
	0.53	3.53 c

[z] Means followed by the same letter are not significantly at Fisher's LSD at $P = 0.05$.

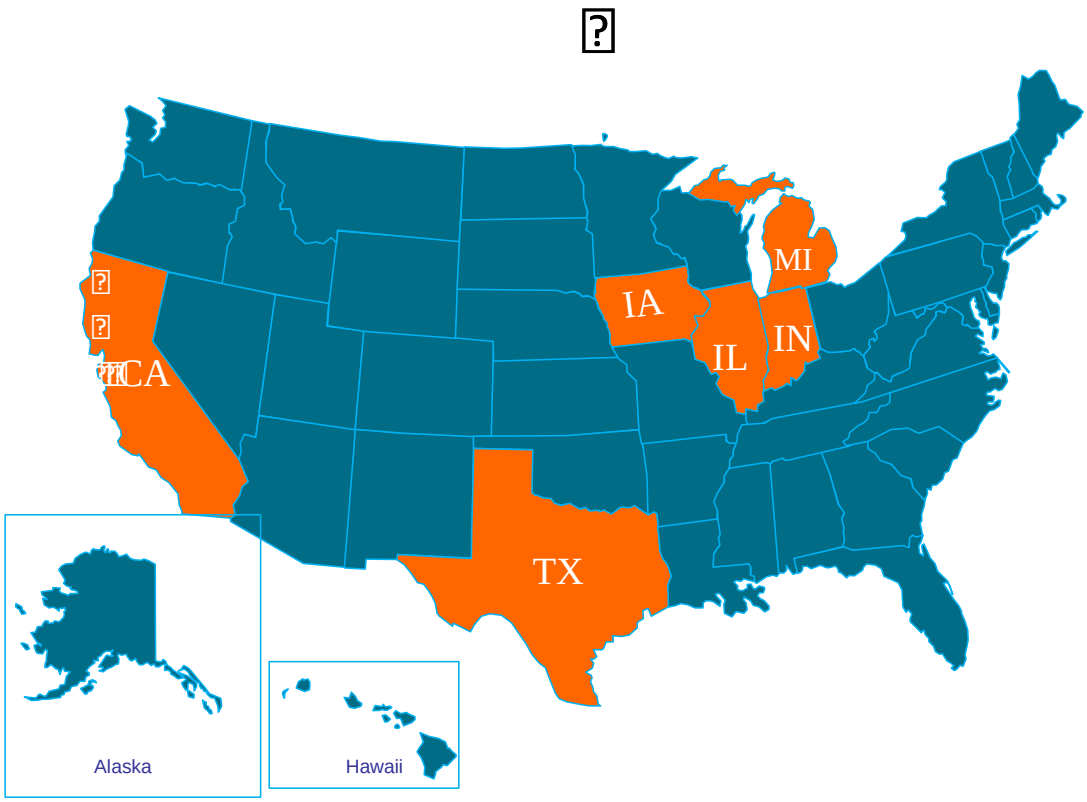

Figure 4.1. States in the United State (in orange) where leaves of cucurbit crops with powdery mildew were collected. CA = California; IA = Iowa; IL = Illinois; IN = Indiana; MI = Michigan; NY=New York; PA=Pennsylvania; TX = Texas; WI=Wisconsin and WA=Washington.

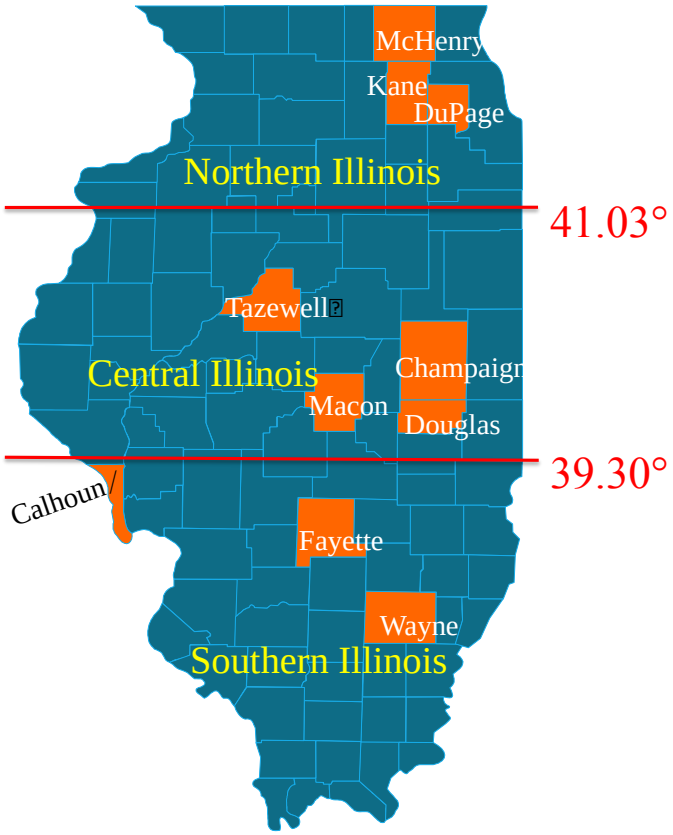

Figure 4.2. Counties in Illinois (in orange) where leaf samples of cucurbit crops with powdery mildew (infected by *Podosphaera xanhii*) in 2014, 2015, and 2016 for the laboratory studies on the sensitivity of powdery mildew fungus to fungicides.

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